THE PHILIPPINE JOURNAL OF SCIENCE

VOL. 49 NOVEMBER, 1932 No. 3

MALARIA AND ANOPHELES RECONNAISSANCE IN THE PHILIPPINES [1]

By RUFUS L. HOLT

Of the United States Army Medical Department Research Board, Manila

and

PAUL F. RUSSELL

Of the International Health Division of the Rockefeller Foundation Chief, Malaria Investigations, Bureau of Science, Manila

SEVEN PLATES AND ONE TEXT FIGURE

INTRODUCTION

The inception of this project was in November, 1930, and resulted from the request of the Headquarters, Philippine Department, United States Army, for more-definite information with regard to the prevalence of malaria in order that maneuvers

[1] The surveys and laboratory studies on which this paper is based were made under the joint auspices of the Medical Department Research Board of the United States Army, the International Health Division of the Rockefeller Foundation, and the Bureau of Science of the Philippine Islands.

The authors are very greatly indebted to Dr. W. V. King, of the United States Bureau of Entomology, who is classifying the anophelines of the Philippines under the sponsorship of the International Health Division of the Rockefeller Foundation. All mosquito collections, after our identifications, were given to Doctor King for his collection. He has very kindly checked the identifications, especially of the *minimus-funestus* group.

The authors are no less indebted to Col. H. H. Rutherford, Department Surgeon, Philippine Department, for his great interest and support in the accomplishment of this work.

They are also indebted to Maj. John H. Kintner, Veterinary Corps, United States Army, for valuable assistance rendered in the survey of

might be held in certain areas with minimum danger from infection by malaria. Other surveys followed in the order shown in Table 1.

TABLE 1.—*Itinerary and distance of reconnaissance surveys.*

Month and year.	Region.	Distance traveled.
		Km.
November, 1930	Bataan, east coast	272
December, 1930	Misamis	2,260
May, 1931	Corregidor and outposts	81
July, 1931	Bataan, central and west coast	337
July and August, 1931	Zambales	374
September and October, 1931	Kabasalan, Zamboanga	2,565
October, 1931	Bataan, east central	248
Do	Pampanga and Tarlac	328
November, 1931	Tayabas	543
December, 1931	Laguna and Batangas	400
Do	Mountain Province	715
January, 1932	Ilocos, La Union, Pangasinan, and Zambales	1,722
January and February, 1932	Mindanao and Sulu	4,597
February, 1932	Visayan and Bicol provinces	2,046
March, 1932	Northeastern provinces, Luzon	2,184
January to March, 1932	Laguna	538
March, 1932	Cavite and Rizal	418
Do	Visayas	2,180
Do	Palawan and Balabac	2,145
Various dates	Miscellaneous (including Baguio)	600
Total		24,553

In Table 1 are given the dates of surveys, places visited, and distances traveled in kilometers. It will be noted that the total distance was 24,553 kilometers. Means of transportation included foot, carabao carts, riding horses, various horse-drawn

Corregidor and its outlying posts, as well as assistance rendered in the completion of data.

Thanks are due to Loyd Stevens, sergeant, Medical Department, United States Army, Andres Nono, chief field inspector, malaria investigations, and Domingo Santiago, field inspector, malaria investigations. Further, we would express our thanks to the Director of the Bureau of Education and to many of the division superintendents and their teachers, who made possible spleen palpations in the schools and who at all times gave us their hearty coöperation. We are also grateful to the Director of the Bureau of Commerce and Industry who arranged for us to make one inspection trip on the cutter *Bustamante*. For data regarding altitudes we are indebted to the Director of the Bureau of Forestry. At various times officials of the Philippine Health Service lent their coöperation, for which we make due acknowledgment. Finally, we would acknowledge our indebtedness to the Director of the Bureau of Science for his assistance in planning this paper and in its publication.

vehicles, motor trucks and cars, bancas, launches, steamers, Government cutters, and railways. Table 2 lists all places in which spleen surveys and/or *Anopheles* collections were made.

TABLE 2.—*Places visited on malaria reconnaissance.*

Abra Province.
- Bangued.
- Palao.

Albay Province.
- Bogtong.
- Daraga.
- Guinobatan.
- Legaspi.
- Mauraro.

Antique Province.
- Pandan.

Bataan Province.
- Abucay.
- Bagac.
- Balanga.
- Cabcaben.
- Corregidor.
- Cupang.
- Dinalupihan.
- Hermosa.
- Limay.
- Mariveles.
- Orani.
- Orion.
- Samal.
- Sisiman.

Batangas Province.
- Cuenca.
- Batangas.
- Ibaan.
- Rosario.
- Sabang.
- Tuy.

Bulacan Province.
- Baisa.
- Malolos.
- Marilao.

Bohol Province.
- Agricultural Colony.
- Balilihan.
- Calape.
- Carmen.
- Leon.
- Tagbilaran.

Cagayan Province.
- Aparri.
- Gattaran.
- Iguig.
- Tuguegarao.

Camarines Sur Province.
- Lagonoy.
- Naga.
- Pasacao.

Capiz Province.
- Buntog.
- Capiz.
- New Washington.

Cavite Province.
- Indang.
- Naic.
- Ternate.

Cebu Province.
- Cebu.
- Labangon.
- Mambaling.
- Mandaue.
- Tabunoc.
- Toledo.

Davao Province.
- Davao.
- Katitipan.
- Matina.
- Mintol.
- Pakiputan.
- Talomo.
- Tugbok (Gulanga).

Ilocos Norte Province.
- Bacara.
- Badoc.
- Bangui.
- Batac.
- Laoag.
- Paoay.
- San Mateo.

Ilocos Sur Province.
- Bantay.
- Cabugao.

Ilocos Sur Province—Continued.
- Suyo.
- Vigan.

Iloilo Province.
- Iloilo.
- Jaro.
- Leon.
- Passi.

Isabela Province.
- Echague.
- Ilagan.

Laguna Province.
- Bay.
- Biñan.
- Cabuyao.
- Calamba.
- Calauan.
- Dayap.
- Lilio.
- Longos.
- Los Baños.
- Luisiana.
- Lumban.
- Mabitac.
- Magdalena.
- Majayjay.
- Nagcarlan.
- Paete.
- Pagsanjan.
- Pila.
- Santa Cruz.
- San Juan.
- San Pablo.
- Santa Maria.
- Santa Rosa.
- Siniloan.

Lanao Province.
- Kolambugan.

La Union Province.
- Bauang.
- Burgos.
- Saitan.

Leyte Province.
- Carigara.

TABLE 2.—*Places visited on malaria reconnaissance*—Continued.

Leyte Province—Ctd.
- Talibong (Biliran Island).

Manila, City of.
- Malacañang.
- Malate.
- Maypajo.

Masbate Province.
- Masbate.
- Tagba.

Mindoro Province.
- Camboag (San Jose).
- Caminawit (Mangarin).
- San Jose.

Misamis Province.
- Cagayan.
- Gingoog.

Mountain Province.
- Baguio.
- Cervantes.
- Hapao.
- Loo.
- Tawan.

Nueva Ecija Province.
- Bongabon.
- Cabiao.
- Cabu.
- Puncan.
- San Jose.

Nueva Vizcaya Province.
- Bagabag.
- Bambang.
- Bayombong.
- Rosario.
- Santa Fe.

Occidental Negros Province.
- Bacolod.
- Isabela.
- Murcia.
- San Carlos.

Oriental Negros Province.
- Dumaguete.

Palawan Province.
- Baja Point (west coast).
- Balabac.
- Coron.
- Culion.
- Cuyo.
- Iwahig.
- Puerto Princesa.

Pampanga Province.
- Del Carmen.
- Floridablanca.
- Fort Stotsenburg.
- La Mitra.
- Porac.

Pangasinan Province.
- Alaminos.
- Infanta.
- Lingayen.
- Mabini (Balincaguin).
- Malasiqui.
- Urdaneta.

Rizal Province.
- Antipolo.
- Las Piñas.
- Navotas.
- Parañaque.
- San Francisco del Monte.
- San Juan del Monte.

Samar Province.
- Allen.

Sorsogon Province.
- Bulusan.
- Putiao.
- Rizal.
- Sorsogon.

Sulu Province.
- Astorias.
- Bato-bato.
- Bongau.
- Jolo.
- Lapac.

Sulu Province—Ctd.
- Lugus Island.
- Maimbung (Jolo).
- Manubul Island.
- Pantao (Jolo).
- Siasi.
- South Ubian.
- Tabo-tati (Jolo).
- Tandu Bato (Jolo).
- Tandu Patong (Jolo).

Tarlac Province.
- Luisita.
- San Miguel.

Tayabas Province.
- Atimonan.
- Candelaria.
- Gumaca.
- Lucban.
- Lucena.
- Malicbuy.
- Mauban.
- Pagbilao.
- Sampaloc.
- Sariaya.
- Tayabas.
- Tiaong.

Zambales Province.
- Candelaria.
- Castillejos.
- Iba.
- Olongapo.
- San Antonio.
- San Felipe.
- San Marcelino.
- San Narciso.
- Santa Rita.
- Subic.

Zamboanga Province.
- Kabasalan.
- Limajon (east coast).
- Mercedes.
- Talungatung.
- Tumaga.
- Zamboanga.

A number of malaria and *Anopheles* surveys have been reported from the Philippines. The first and most extensive of previous reports was that of Barber et al.(1) These observers visited fourteen provinces making collections of *Anopheles* larvæ. They also reported a total of 4,113 blood-smear examinations in children with a parasite index of 11.0 per cent and a total of 5,426 spleen examinations with a splenic index of 13.3 per cent.

Other surveys of special interest have been reported by Nichols,(2) Musgrave et al.,(3) Padua,(4, 5, 6) Manalang,(7, 8) Tiedeman,(9) Ejercito,(10,11) Mieldazis,(12) Ejercito and Laurel,(13) Holt and Russell.(14) Some of the information published in the last-cited report is included in this. G. F. Lull,(15) who was Medical Advisor to the Governor-General at the time of his report, gives the following figures for the mortality of malaria in the Philippine Islands (Table 3).

TABLE 3.—*Malaria in the Philippines, from Lull.*

Year.	Deaths.	Death rates for 100,000 population.	Year.	Deaths.	Death rates for 100,000 population.
1904	33,655	588.34	1918	38,322	411.42
1905	41,446	661.89	1919	37,726	398.00
1906	23,973	472.64	1920	29,653	308.00
1907	22,610	352.97	1921	28,407	281.78
1908	23,487	364.90	1922	27,196	257.74
1909	25,751	378.12	1923	24,142	218.14
1910	26,359	359.68	1924	26,740	232.94
1911	28,181	370.15	1925	24,329	218.39
1912	27,229	345.99	1926	24,318	210.63
1913	18,526	229.62	1927	19,520	166.32
1914	20,285	246.92	1928	15,925	133.78
1915	24,826	297.21	1929	15,332	127.02
1916	26,088	307.19	1930 [a]	15,144	123.61
1917	29,074	317.75	1931 [b]	11,787	94.89

[a] Figures for 1930 and 1931 were supplied by the Director of the Philippine Health Service.

[b] Figures for 1931 are provisional.

Ash,(16) in 1922, conducted a spleen survey along the eastern shore of Bataan examining 662 children in the area between Mariveles and Guagua, finding 128 children with enlarged spleens, all of which were south of Orion, Bataan.

Simmons and St. John(17) made a malaria survey on Corregidor in July, 1928, during which they examined 1,740 children,

finding a splenic index of 3.6 per cent with 74.2 per cent correlation between positive spleens and parasites in the blood. The same authors with Reynolds(18) conducted a similar survey at Fort Stotsenburg, Pampanga, examining 993 children and finding 2.2 per cent with enlarged spleens. Of the positive spleen cases, 96 per cent were found to be harboring malaria parasites in the blood.

In view of the fact that none of the above-mentioned surveys included a majority of the provinces of the Philippines and because there has recently been an extensive revision in the classification of the local species and varieties of *Anopheles*, it seemed highly desirable to make a reconnaissance survey, first to determine in general the distribution of the various anophelines throughout the entire Archipelago, and second, to find out whether or not malaria is an island-wide problem. The present survey required eight months of nearly continuous travel in addition to numerous shorter surveys and gathered data from forty-one of the forty-eight provinces.

The survey party usually consisted of the authors, each with an assistant, but at times numbered eight persons. It was impossible, in the time available, to visit Romblon, Marinduque, and Camarines Norte Provinces and to penetrate the central portions of Mindanao, Mountain Province, Samar, and Leyte. Our itinerary is given in Table 1 and a list of places visited in Table 2.

GENERAL CONSIDERATIONS

The Philippine Islands extend from Sibutu, latitude 4° 40′ north, to Y'Ami, latitude 21° 5′ north, or about 1,100 miles. The Archipelago comprises 7,083 islands of which about 2,500 are named. The area of the group is about 114,000 square miles, a little less than the British Isles. Luzon and Mindanao comprise more than half of the area of the total. About 13,000,000 people inhabit the Islands according to latest official estimates.

Climate, rainfall, and average temperature conditions in the Philippines are shown by Plates 1 and 2, and no further discussion of these subjects is necessary in this paper.

As Craig(19) first pointed out, malaria in the Philippines is found in the foothills. As will be seen from Table 4, our experience bears this out. We have not found malaria on the coastal plains, on the plateaus, or in the mountains above 2,000 feet; but except for this fact that malaria transmission apparently does not occur above 2,000 feet, altitude per se, we believe,

has little influence on the prevalence of malaria. It is change in altitude which has a direct and fundamental influence on malaria in the Philippines (see Table 4).

TABLE 4.—*Showing noneffect of altitude per se on malaria rates in selected places.*

Altitude.	Town and province.	Spleen index.
Feet.		
20 or less	Navotas, Rizal	3.0
	Abucay, Bataan	4.9
	Legaspi, Albay	6.4
	Dinalupihan, Bataan	30.6
	Siniloan, Laguna	33.7
	Bangui, Ilocos Norte	41.3
	Gattaran, Cagayan	61.8
30–100	Iguig, Cagayan	5.7
	Pagbilao, Tayabas	7.8
	Cabugao, Ilocos Sur	33.7
	Santa Maria, Laguna	57.0
100–200	San Miguel, Tarlac	6.0
	La Mitra, Tarlac	8.0
	Castillejos, Zambales	10.5
	Palao, Abra	22.4
	Echague, Isabela	38.1
300–500	Rosario, Batangas	13.7
	San Jose, Mindoro	26.5
	Suyo, Ilocos Sur	35.0
500–1,500	Cuenca, Batangas	14.0
	Saitan, La Union	22.8
Over 2,000:	Bagabag, Nueva Vizcaya	90.0
3,250	Hapao, Mountain	0.0
5,150	Loo, Mountain	0.0

SPLEEN RECONNAISSANCE

It should not be necessary at this date to write an apologia for the use of splenic enlargement as an index to the distribution and prevalence of malaria in a community, but doubt still creeps into the literature and has been locally expressed. Therefore, a brief review of the situation seems to be indicated.

Since the time of Sydenham,(23) and doubtless before, the relation between enlarged spleen and ague has been known. For at least eighty-five years this relationship has been used as a clue to the presence and prevalence of malaria in a community. Dempster(24) in India appears to have been first to show that this positive correlation between a large spleen and malarial fever is of practical value as a measure of malarial endemicity. He wrote that localities "free from splenic disease may be

confidently selected as a good position for European and native troops." He concluded that "however healthy a locality may appear in other respects, if its native inhabitants are generally afflicted with organic disease of the spleen, there, beyond all doubt, does much malaria exist * * *." Hundreds of reported spleen surveys from every continent have amply borne out this clear-cut statement of 1847.

The spleen index is rapidly taken, causes little disturbance, and is probably more reliable as indicating the average prevalence of malaria than the blood index. We have seen no evidence that the spleen index is any less useful as an indication of malaria distribution and prevalence in the Philippines than elsewhere. We agree with Christophers(25) that "the ease with which it can be ascertained over large areas and for large numbers makes it a unique index in the measurement of disease, and to the malariologist it is one of the most precious assets of his science."

In view of attempts to depreciate the value of the spleen survey it must be kept in mind that malaria is not easily and accurately measured by any test. The plasmodia have the habit of secreting themselves in the recesses of spleen and bone marrow so that a negative blood smear may mean very little. As Manson(26) was wont to say, "what is found in the peripheral blood in malaria represents only an overflow from much greater visceral happenings. The splenic enlargement is one result of such happenings and is as important a measure of the malaria incidence as the microscopical examination of one thin film of blood."

Other references could be quoted; for example, that of Ross, Christophers, and Perry(27) who, on the basis of very extensive experience, wrote that the spleen rate is the "most readily and extensively applicable, and at the same time the most reliable measure of the amount of malaria in a community * * *."

As regards the Philippines, the most extensive spleen survey in the past was that of Barber et al.(1) who made 5,426 spleen and 4,646 blood smear examinations in fourteen provinces. They found a parasite index of 11 per cent and a spleen index of 13.3 per cent. Of the 3,686 children who had both spleen and blood examinations, 7.5 per cent were positive for parasites and 12.4 per cent for splenic enlargement. In communities in which at least one parasite-positive case was found, that is in malarious places, there were 1,883 spleen-negative cases among children

and of these 7.7 per cent were parasite-positive. The authors state further, "of approximately 569 cases presenting enlarged spleens of whom the blood was examined, 164 or 28.8 per cent, were positive for parasites. It is well known that cases exhibiting splenomegaly, known to be the result of malaria, often fail to show parasites in the blood."

The authors conclude, "while a larger series than ours may be needed to solve this question, we believe that a spleen rate over 10 indicates present or past malaria in the most, if not all the communities of the Philippines. However, we should not recommend that the malarial survey of a locality should rest on the spleen examination."

Except that we believe spleen rates of more than 5 to have malarial significance, we agree, and feel that in any given locality accurate measurement of malaria requires both spleen and blood tests. In a reconnaissance such as we are reporting we feel confident that the spleen test alone is a fair indication of the presence or absence of malaria. We are not using it as more than a broad measure of local intensity and have not given any totals by provinces, believing that such totals should only be given where a province has been thoroughly surveyed.

It is well known that other diseases cause splenic enlargement and that the examiner must, therefore, be on the lookout for such acute infections as septicæmia, typhus and typhoid fevers, measles, chicken pox, mumps, erysipelas, acute miliary tuberculosis, tuberculous peritonitis, cerebrospinal meningitis, Malta fever, smallpox, diphtheria, scarlet fever, relapsing fever, and plague. These diseases are listed by Boyd(28) as producing acute enlargement of the spleen. This author further states that chronic enlargement, other than malarial, is observed in congenital syphilis, schistosomiasis, kala azar, leukemia, splenic anæmia, Gaucher's disease, malignant neoplasms, and familial jaundice. Lesser degrees of enlargement are present in cirrhosis of the liver, rickets, pernicious anæmia, passive congestion, and acute portal obstruction.

This is an imposing list, but in practice in the Philippines in spleen examinations in schools and public places—that is, not in hospitals, dispensaries, or homes—confusion may arise only from measles, chicken pox, and, in some regions, schistosomiasis. During our survey we encountered both measles and chicken pox. With regard to measles and chicken pox our experience has been in line with the reports by Boyd.(28) Splenic enlargement in

these diseases does not become chronic. By the time the rash has faded, the spleen is usually not palpable even on deep inspiration. In our tables these cases, giving evidence of recent chicken pox or measles, have been excluded.

According to a recent survey by Tubangui,(29) there are foci of schistosomiasis in Leyte. We found no cases in our brief visits at the northern end of this island and saw none elsewhere, but the disease unquestionably is fairly prevalent in the areas visited by Tubangui and must be kept in mind in spleen surveys in the Philippines.

In technic of examination we have used wherever possible the more delicate method of Darling.(30) The child is placed in a recumbent position with the thighs and legs flexed. The clothing is loosened so that the hand of the examiner can be placed upon the bare skin of the abdomen in the region of the spleen at the costal margin. Standing on the child's right, the examiner gently explores the upper left quadrant of the abdomen with the finger tips, feeling for an enlarged spleen. If the spleen is not palpable, the child is instructed to take a deep breath. The tips of the fingers of the examiner's right hand are held at and just below the costal margin. As the child breathes deeply, slight pressure is made with the fingers and sometimes a spleen, pushed down by the diaphragm, becomes palpable.

Having palpated a spleen it may be classified as to size according to one of several systems. These vary from the simple but satisfactory finger's breadths of the earlier malariologists to the latest method of triangulation of the position of the apex of the spleen by measurement of its distance in centimeters from the umbilicus and from the median line of the body, with corrections for the age and size of the child. This refined technic of Christophers(31) is particularly useful in quantitative and comparative studies of community spleen mass and is certainly the most accurate of all devices for recording spleen sizes. It has no place, however, in a reconnaissance, for it is a burdensome and slow process. Furthermore, in the Philippines, it could usually be applied only to boys. There has been no objection from girls or their parents, to a simple palpation of the child's spleen, hand on skin, without exposure, in the schoolroom. Exposure usually would not be tolerated. Therefore, in our survey we have not attempted meticulous measurements. In a few localities it was necessary to examine children in the standing position because of their shyness. These exceptions are duly noted in the table which summarizes the results.

We have classified our spleens in six groups as follows, after Boyd:(28)

Spleen 0. Spleen not palpable.
Spleen P. D. I. Palpable only on deep inspiration.
Spleen 1. Palpable at the costal margin on normal respiration.
Spleen 2. Palpable, extends halfway to the umbilicus.
Spleen 3. Palpable, extends to the umbilicus.
Spleen 4. Palpable, extends beyond the umbilicus (see fig. 1).

The interpretation of these groups is of course open to question. The negative group undoubtedly includes some cases of malaria. This is evident not only from the discussion above but from the study of Oudendal.(32) This author, in a series of cases, made both ante- and post-mortem spleen examinations with the results shown in Table 5.

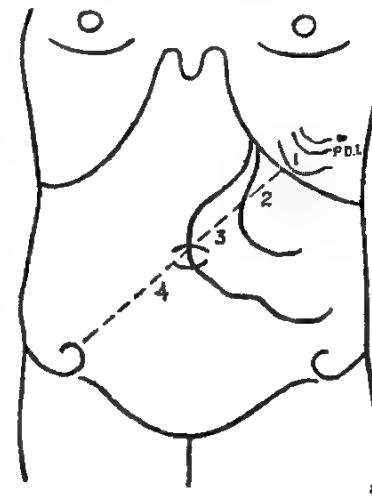


FIG. 1. Diagram of spleen sizes according to Boyd.

Oudendal concluded logically that many factors influenced the palpability of the spleen, such as its length, width, thickness, consistency, and weight. Also the position of the diaphragm, the status of the abdominal wall and abdominal cavity, the direction of the longitudinal splenic axis, and the deftness of the palpating hand. So the absence of a palpable spleen cannot always be considered conclusive evidence of an absence of malaria.

TABLE 5.—*Spleen weights from Oudendal.*

Spleen weight post mortem.	Number examined.	Palpable ante mortem.	
		No.	Per cent.
g.			
110–250	165	24	14.5
250–400	103	41	37.7
400–870	87	51	58.7
870–2,147	28	27	96.4

As to the significance of the spleens palpable on deep inspiration, doubt has been expressed by some and confidence by others. Darling(30) was the first to use this class of spleens in the measurement of malaria. He believed that in regions of light endemicity the spleens just palpable on deep inspiration were of some value as an indication of malaria, although he recognized that this group must be used with discrimination as it undoubtedly includes spleens not malarious. But he believed that when intelligently interpreted the smallest palpable spleens as a class contributed definitely to the malaria picture.

One of us (P. F. R.) assisted in some studies in the Southern United States which seem to have borne out these contentions. As reported by Darling,(33) these showed first, that the palpable-on-inspiration spleens in negro children may show a high percentage of positive blood smears, as seen in Table 6.

TABLE 6.—*Spleens in negro children, from Darling.*

	Spleen group.					
	Negative.	Palpable on inspiration.	Palpable.	One finger's breadth.	Two fingers' breadth.	Three fingers' breadth.
Number	260	110	65	32	56	47
Positive blood films in each spleen group:						
Number	25	60	45	26	43	43
Per cent	9.6	54.5	69.2	81.3	76.8	91.5

Secondly, these studies showed that the palpable-on-inspiration spleens are affected by quinine. Darling estimated the weights of the different class spleens as follows:

	g.
Negative spleen	75
Palpable on inspiration	90
Palpable	105
One finger's breadth below costal margin	125
Two fingers' breadth below costal margin	150
Three fingers' breadth below costal margin	180
To umbilicus	220

These estimates were based in part on Bean's(34) studies. Using these estimates, Table 7 illustrates the effect of the Bass(35) standard quinine treatment on spleen size in forty-five negro children.

TABLE 7.—*Effect of Bass quinine treatment on spleen size in negro children.*

	Spleen size.					
	Palpable on inspiration.	Palpable.	One finger's breadth.	Two fingers' breadth.	Three fingers' breadth.	Umbilicus.
Number of cases	11	12	7	11	3	1
Weight before treatment—g.	11×90	12×105	7×125	11×150	3×180	1×220
	990	1,260	875	1,650	540	220
Weight after 8 weeks' treatment—g.	945	1,140	660	1,145	375	90
Change in weight—g.	— 45	—120	—215	—505	—165	—130
Change in weight per person—g.	— 4	— 10	— 30	— 45	— 55	—90

In a control group of twenty-five negro children who had no treatment but were examined each time there were the changes shown in Table 8.

TABLE 8.—*Spleen size in untreated negro children.*

	Spleen size.					
	Palpable on inspiration.	Palpable.	One finger's breadth.	Two fingers' breadth.	Three fingers' breadth.	Umbilicus.
Number of cases	8	8	5	6	1	0
Weight before treatment—g.	720	840	625	900	180	
Weight after ten weeks (no treatment)—g.	720	835	480	905	150	
Change in weight—g.	0	— 5	—245	— 5	— 30	
Change in weight per person—g.	0	—0.6	— 49	—0.8	— 30	

For a complete analysis of these experiments see in addition to Darling,(33) also Collins.(36)

Another reason for believing that the palpable-on-inspiration spleens have malarial significance is the fact that they are not found to any extent in nonmalarial localities, but they are found in malarious places. The more malaria the greater the numbers of palpable-on-inspiration spleens. For example, one of us(37) examined one hundred twenty-one boys in Georgetown on Penang Island and found only four palpable-on-inspiration spleens, and none larger. In Permatang Imas Laut, where malaria was hyperendemic, twenty-eight palpable-on-inspiration spleens were found among a total of sixty enlarged spleens in sixty-six examinations. The largest spleen was of pelvic size, two were at the umbilicus, and nineteen were from one to four fingers' breadth in size.

In the present survey it will be noted, as shown in Table 9, that in Manila, Lingayen, Cebu, and Navotas, where malaria is not endemic, only eleven palpable-on-inspiration spleens were found in a total of four hundred three examinations in which there were only twelve enlarged spleens altogether, including those palpable on inspiration. In Bambang, Bangui, San Jose, and Bulusan, where malaria is endemic, thirty-three palpable-on-inspiration spleens were found among a total of one hundred twenty-seven enlarged spleens in two hundred fifty-one examinations.

Our present series of cases having both blood smear and spleen palpation is too small to carry much weight. However, it may be noted that of fourteen individuals having palpable-on-inspiration spleen, 9, or 64.3 per cent, also had positive blood smears.

We believe that this group of palpable-on-inspiration spleens in the Philippines has real value in a malaria reconnaissance. There are regions, like Ilagan, Isabela Province, where during the dry season the small streams become completely dry and practically speaking, malaria transmission ceases. During the wet season typical *A. minimus* breeding places abound and there is a "fever season." Our examinations were made in March when no anopheles larvæ could be found, in the middle of the dry period. We found fourteen palpable-on-inspiration spleens in eighty-four children. Only four other spleens were palpated. One was class 2 and three were class 1. Without the fourteen spleens in the palpable-on-inspiration group the splenic index is 4.5 per cent. With the small spleens included the index is 21.7 per cent. The former figure by itself is of no malarial significance yet includes definitely malarious spleens and is in a locality which has a definitely malarious season. The latter figure of 21.7 per cent is probably a much more reliable index as to the malarial status of Ilagan because it includes the residual spleens from the last malarial season. These small spleens, where numerous, probably represent chronic quiescent infections, although they may occasionally be indicative of recent but mild attacks, and of course may sometimes be without significance.

As to the significance of the larger spleens we are inclined to believe that MacDonald's(38) theories apply in a general way to the Philippines. Spleens of size 1 probably represent either recent active infections or chronic quiescent malaria. Size 2 spleens represent perhaps reinfections or relapses in those long

infected. Sizes 3 and 4 are due to frequent reinfections in individuals whose tolerance is not yet well developed.

As to the belief that splenic enlargement is constant in tertian and quartan infections but variable in æstivoautumnal malaria, and that the two former produce a greater enlargement than the latter, we have insufficient data to express an opinion. See Stephens and Christophers.(39)

There was a total of 1,404 enlarged spleens among the 7,810 individuals examined. This is an index of 17.9 per cent. If we assume a population of 13,000,000 (this is the usual assumption at the present time in the various Government bureaus) this indicates roughly a malaria morbidity of 2,300,000. If we assume the usual prognosis, with a mortality of 1 per cent, we could expect a death rate from malaria in the Philippines of around 23,000. We believe this figure to be approximately correct. Undoubtedly, many deaths are incorrectly attributed to malaria, but it is equally true that many malaria deaths are not so reported.

The map shown as Plate 3 has been prepared to show in general these spleen findings. It will be noted that we have arbitrarily classified our spleen indices as follows:

Per cent.		
5 or less	Not significant	N. S.
6–10	Faint endemicity	F.
11–20	Mild endemicity	M.
21–30	Moderate endemicity	M.
31–50	Severe endemicity	S.
51 or more	Hyperendemicity	H.

Tables 9 and 10 represent, respectively, the findings by locality and size of spleen in the first instance, and age, sex groups, and size of spleen in the second instance. In these tables are included figures by Ash on Mariveles, Bataan, by Simmons, St. John, and Reynolds on Fort Stotsenburg, Pampanga, and by George C. Dunham on Bongau and Batobato, Sulu. The figures by Ash were compiled in 1922, by Simmons, St. John, and Reynolds, in 1929, and by Dunham, in 1932. Since the preparation of these tables we have secured our own figures on Mariveles. Of forty-eight children examined, we found only four palpable-on-inspiration spleens, or a positive per cent of 8.4, as against the findings by Ash of 31.6 per cent. In addition, we examined eleven children at Cabcaben, Bataan, and found one No. 3 spleen and five No. 1 spleens, a positive rate of 54.5 per cent.

TABLE 9.—*Spleen survey. By locality and size of spleen.*

Province and locality.	Population.	T.	Examined.	Negative.	Size of spleen.					Positive.	
					P. D. I.	1	2	3	4	Number.	Per cent.
Abra:											
Bangued	13,892	L	94	63	13	14	3	1	----	31	33.0
Palao	339	L	76	59	6	9	2	----	----	17	22.4
Albay:											
Bogtong	1,306	L	46	39	5	1	1	----	----	7	15.2
Legaspi	6,055	L	93	87	4	2	----	----	----	6	6.4
Mauraro	2,891	L	47	39	5	3	----	----	----	8	17.0
Antique:											
Pandan	16,226	L	115	97	9	9	----	----	----	18	15.7
Bataan:											
Abucay	7,347	L	102	97	3	----	1	1	----	5	4.9
Balanga	8,141	L	174	156	12	----	1	5	----	18	10.3
Corregidor	8,000	L	107	90	11	3	2	1	----	17	16.0
Dinalupihan	4,107	L	36	25	7	----	1	3	----	11	30.6
Hermosa	3,150	L	127	118	6	----	1	2	----	9	7.0
Limay	3,514	L	56	43	8	----	5	----	----	13	23.2
Mariveles	2,816	L	57	39	----	----	----	----	----	18	31.6
Orani	6,336	L	144	136	6	----	2	----	----	8	5.6
Orion	7,887	L	152	134	11	----	5	2	----	18	11.8
Samal	5,231	L	107	100	7	----	----	----	----	7	6.5
Sisiman	116	L	14	4	----	2	5	1	2	10	71.3
Batangas:											
Batangas	41,089	L	86	80	6	----	----	----	----	6	7.0
Cuenca	7,106	L	57	49	5	3	----	----	----	8	14.0
Rosario	22,174	L	51	44	5	2	----	----	----	7	13.7
Tuy	5,847	L	104	78	13	9	2	2	----	26	25.0

Cagayan:											
Aparri	20,603	S	24	22	1	1				2	8.3
Gattaran	7,009	S	34	13	1	11	4	4	1	21	61.8
Iguig	5,413	S	52	49	1	2				3	5.7
Tuguegarao	19,298	S	54	47	5	2				7	13.0
Camarines Sur:											
Lagonoy	12,681	L	57	46	6	5				11	19.3
Naga	9,396	L	56	50	4	2				6	10.7
Pasacao	2,308	L	71	46	13	8	2	2		25	35.2
Capiz:											
New Washington	24,458	L	89	87	2					2	2.3
Cebu:											
Cebu	65,502	L	89	87	2					2	2.3
Davao:											
Davao	13,300	L	82	79	2	1				3	3.7
Katitipan	80	S	21	21							0.0
Mintol	146	L	31	28	2	1				3	9.7
Tugbok	214	S	41	37		4				4	10.0
Ilocos Norte:											
Badoc	17,598	L	76	53	6	8	7	2		23	30.2
Bangui	10,654	L	80	47	7	18	5	3		33	41.3
Laoag	38,469	L	131	94	15	13	6	3		37	28.2
Ilocos Sur:											
Cabugao	12,025	L	95	63	11	15	4	2		32	33.7
Suyo	638	L	40	26	3	10		1		14	35.0
Vigan	17,765	L	52	48	3	1				4	7.7
Isabela:											
Echague	17,104	L	42	26	10	6				16	38.1
Ilagan	23,279	L	84	66	14	3	1			18	21.7
Laguna:											
Calauan	2,832	L	82	52	12	11	2	5		30	36.6
Magdalena	3,032	L	78	48	15	8	3	3	1	30	38.3
Pagsanjan	7,538	L	51	44	6	1				7	13.7
Santa Cruz	14,156	L	53	47	2	4				6	11.3

TABLE 9.—*Spleen survey. By locality and size of spleen*—Continued.

Province and locality.	Population.	T.	Examined.	Negative.	Size of spleen.					Positive.	
					P. D. I.	1	2	3	4	Number.	Per cent.
Laguna—Continued.											
Santa Maria	540	L	37	16	2	7	5	5	2	21	57.0
Sinlloan	3,918	L	83	55	11	10	4	2	1	28	33.7
La Union:											
Bauang	12,952	L	50	43	7					7	14.0
Burgos	500	L	51	40	6	4	1			11	21.5
Saitan	550	L	63	48	6	8	1			15	23.8
Leyte:											
Carigara	17,558	L	86	72	7	6	1			14	16.3
Talibong (Biliran Island)	554	L	32	28	3	1				4	12.5
Manila:											
American School	285,306	L	40	39	1					1	2.5
Malate School		L	75	72	3					3	4.0
Masbate:											
Masbate	10,821	L	99	92	5	2				7	7.0
Mindoro:											
Camboag	60	S	7	0			1	2	4	7	100.0
Caminawet (Mangarin)	487	L	18	10	3	2	3			8	44.4
San Jose	7,703	L	78	38	9	13	5	6	7	40	51.3
Mountain Province:											
Hapao	1,000	S	10	10						0	0.0
Hungduan	1,000	S	3	3						0	0.0
Loo	2,000	S	51	51						0	0.0
Taiwan	500	S	15	15						0	0.0
Nueva Ecija:											
Bongabong	4,434	L	52	26	10	13	2	1		26	50.0
Cabu	209	L	47	35	6	4	2			12	25.5

Puncan	496	L	47	14	10	14	6	3		33	70.2
San Jose	834	L	49	III	9	4				13	26.5
Nueva Vizcaya:											
Bagabag	3,730	L	40	4	8	10	9	6	3	36	90.0
Bambang	2,564	L	47	8	10	17	6	3	3	39	83.0
Bayombong	5,627	L	40	7	15	12	2	3	1	33	82.5
Santa Fe	625	S	21	3	2	9	2	3	2	18	85.7
Pampanga:											
Floridablanca	8,477	L	50	47	2	1				3	6.0
Fort Stotsenburg	9,000		993	971						22	2.2
La Mitra	5,000	L	50	46	2	2				4	8.0
Porac	8,000	L	50	41	3	4		2		9	18.0
Pangasinan:											
Alaminos	14,353	L	54	44	3	6	1			10	18.5
Infanta	5,613	L	47	42	2	2		1		5	11.1
Lingayen	22,750	L	100	97	2	1				3	3.0
Mabini (Balincaguin)	4,760	L	50	36	6	6	2			14	28.0
Rizal:											
Navotas	13,454	L	99	96	3					3	3.0
Samar:											
Allen	8,497	L	47	34	6	5	1	1		13	27.7
Sorsogon:											
Bulusan	8,046	L	46	31	7	8				15	32.6
Putiao	614	L	42	37	5					5	12.0
Rizal	2,224	L	38	28	4	5	1			10	26.3
Sorsogon	16,694	L	94	86	7	1				8	8.5
Sulu:											
Bongau	1,979	L	40	34			3	3		6	15.0
Bato-bato	750	L	35	16			4	14	1	19	54.3
Jolo	5,810	L	74	62	3	8	1			12	16.2
Lapac		L	44	35	8	1				9	20.8
Lugus Island	4,800	L	123	99	11	9	2	2		24	19.5
Maimbung (Jolo)	1,950	L	36	23	7	3	2	1		13	36.1
Manubul Island	900	L	60	50	2	6	1	1		10	16.7
Pantao (Jolo)	220	L	12	7	2	2		1		5	41.7

TABLE 9.—*Spleen survey. By locality and size of spleen*—Continued.

Province and locality.	Population.	T.	Examined.	Negative.	Size of spleen.					Positive.	
					P. D. I.	1	2	3	4	Number.	Per cent.
Sulu—Continued.											
Siasi	245	S	76	66	-------	7	1	1	1	10	13.2
South Ubian Island	4,327	S	78	56	-------	11	4	7	-------	22	28.2
Tayabas:											
Atimonan	18,087	L	56	54	2	-------	-------	-------	-------	2	3.6
Lucban	12,913	L	50	48	1	1	-------	-------	-------	2	4.0
Lucena	12,108	L	49	46	-------	3	-------	-------	-------	3	4.1
Mauban	12,516	L	48	41	-------	2	3	2	-------	7	14.6
Pagbilao	6,879	L	77	71	2	3	-------	1	-------	6	7.8
Sampaloc	3,074	L	56	45	-------	6	4	1	-------	11	19.7
Tayabas	14,983	L	50	27	-------	6	10	7	-------	23	46.0
Zambales:											
Castillejos	4,000	L	38	34	1	2	1	-------	-------	4	10.5
Iba	5,452	L	45	34	5	5	1	-------	-------	11	24.4
San Antonio	5,164	L	56	53	1	1	1	-------	-------	3	5.4
Santa Rita	375	L	35	30	1	2	1	-------	1	5	14.3
Subic	14,159	L	50	46	2	1	1	-------	-------	4	8.0
Zamboanga:											
Kabasalan	2,350	L	144	81	-------	30	20	13	-------	63	43.7
Mercedes	2,765	L	65	54	7	3	-------	1	-------	11	17.0
Tulungatung	675	L	52	43	3	6	-------	-------	-------	9	17.3
Zamboanga	30,798	L	50	47	1	1	-------	1	-------	3	6.0
Total	----------	------	7,810	6,406	529	488	182	135	30	1,404	----------

TABLE 10.—*Spleen-survey totals. By age and sex groups and size of spleen.*

Group.		Examined.	Spleen size.												Total enlarged.	
			Negative.		P. D. I.		1		2		3		4			
Sex.	Age.		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Male	All	3,702	2,948	79.6	276	7.5	272	7.3	106	2.9	81	2.2	19	.5	754	23.7
Do	–1	9	4	4.4					2	22.2	3	33.3			5	55.5
Do	1–4	103	59	5.7	3	2.9	21	20.4	8	7.7	7	6.8	5	4.8	44	42.7
Do	5–9	2,637	2,133	80.9	201	7.6	168	6.4	72	2.7	54	2.0	9	.4	504	19.1
Do	10–19	942	742	78.8	71	7.5	83	8.9	24	2.5	17	1.8	5	.5	200	21.2
Do	20+	11	10	90.9	1	90.9									1	9.1
Female	All	2,855	2,267	79.4	244	8.5	205	7.2	74	2.5	54	1.9	11	.4	588	20.7
Do	–1	3	1	33.3							2	66.6			2	66.6
Do	1–4	89	56	62.9	2	2.2	15	16.9	5	5.6	8	9.0	3	3.4	33	37.1
Do	5–9	2,297	1,837	79.9	212	9.2	151	6.6	56	2.4	34	1.4	7	.3	460	20.0
Do	10–19	466	373	80.0	30	6.4	39	8.4	13	2.8	10	2.1	1	.2	93	20.0
Do	20+															
Total, male and female	–1	12	5	41.7					2	16.6	5	41.6			7	5.9
Do	1–4	192	115	60.0	5	2.6	36	18.7	13	6.8	15	7.8	8	4.2	77	40.1
Do	5–9	4,934	3,970	80.4	413	8.3	319	6.5	128	2.6	88	1.8	16	.3	964	19.5
Do	10–19	1,408	1,115	79.2	101	7.2	122	8.6	37	2.6	27	1.9	6	.4	293	20.8
Do	20+	11	10	90.9	1	9.1									1	9.1
Undetermined age and group		1,050	1,010	96.2											40	3.8
Undetermined age		203	181	89.1	9	4.4	11	5.4	2	.9					22	10.8
All		7,810	6,406	82.0	529	6.8	488	6.3	182	2.3	135	1.7	30	.4	1,404	17.9

These tables show a total of 7,810 children examined with a positive rate of 17.9 per cent, there being 1,404 positives in the total number examined. It will also be noted that the greatest number fell in the age group 5 to 9. This is due to the fact that children in the first and second grades of school were examined in the great majority of instances. Of the 1,408 falling in the age group 10 to 19, but few were over 12 years of age. The population figures are not accurate, since they were taken from the 1918 census report, the latest available. The "T" in Table 9 is the technic of examination, standing or lying. There appears to be but little difference in the rate as between the sexes but there was a higher percentage of positives among the younger age groups in both sexes.

PARASITE STUDIES

In Table 11 are listed the results obtained in the examination of 1,101 thin blood smears taken during our reconnaissance. Active antimosquito and antimalaria work is being carried out at Fort Stotsenburg and Fort Mills only. In no case were the smears taken from selected cases except at Hacienda Mataan where only those cases giving positive histories were selected. It seems evident that all cases at Fort Mills were contracted somewhere else since exhaustive search failed to show the presence of the vectors on Corregidor Island at the time the slides were taken. In the undetermined column are placed those undoubtedly positive but the type of which could not be determined. In all cases slides were examined for thirty minutes unless plasmodia were found sooner. It is evident that *P. malariæ* is rare and that *P. falciparum* and *P. vivax* are common at all places where examinations were made.

We had opportunity to secure but little evidence as to the correlation between spleen-positive and malaria-positive cases. In Table 12 are given the results in 104 cases. In each case a thin smear was used and thirty minutes consumed in examination unless found positive before thirty minutes' study. We realize that many positives were probably missed due to absence of plasmodia in the peripheral circulation. While only 14 palpable-on-inspiration spleens are included, it is striking that 64.3 per cent showed plasmodia in the peripheral blood. As other workers have found, the larger the spleen the more plasmodia positives did we find. In no case did we select for inclusion in the table any person for any other reason except that they had an enlarged

TABLE 11.—*Summary of blood-smear examinations.*

Place.	Date.	Examined.	*Plasmodium vivax.*		*Plasmodium falciparum.*		*Plasmodium malariæ.*		*Plasmodium vivax* and *P. falciparum.*	
			Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Kabasalan, Mindanao	Oct. 1, 1931	493	50	10.2	48	9.7			4	0.8
Gingoog, Mindanao	Dec. 5, 1930	271	56	20.7	50	18.5	4	1.5	11	4.1
Fort Stotsenburg, Pampanga	1930, 1931, 1932	187	5	2.7						
Fort Mills, Corregidor	June 14, 1931	107	14	13.1	4	3.7				
Hacienda Natib, Bataan	July 4, 1931	29	14	48.3	2	7.0	1	3.5		
Hacienda Mataan, Bataan	July, Nov., Dec., 1931	150	30	20.0	39	26.0			5	3.3
Total		1,237	169	13.6	143	11.6	5	0.4	20	1.6

Place.	Date.	*Plasmodium vivax* and *P. malariæ.*		All three parasites.		Undetermined.		Positive.		Negative.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Kabasalan, Mindanao	Oct. 1, 1931							94	19.1	399	80.9
Gingoog, Mindanao	Dec. 5, 1930			1	0.4	3	1.1	73	26.9	198	73.1
Fort Stotsenburg, Pampanga	1930, 1931, 1932							5	2.7	182	97.3
Fort Mills, Corregidor	June 14, 1931							18	16.8	89	83.2
Hacienda Natib, Bataan	July 4, 1931	1	3.5					16	55.2	13	44.8
Hacienda Mataan, Bataan	July, Nov., Dec., 1931							74	49.3	76	50.7
Total		1		1		3	0.3	280	22.6	957	77.4

spleen. No conclusions can be drawn due to the small number of cases, but the table is reproduced in order that it may be included with other findings of the same character by other workers.

TABLE 12.—*Spleen size and blood smears.*

Positive smears.	Spleen size.						
	Not palpable.	P. D. I.	1	2	3	4	Total.
Examined	79	14	43	27	14	6	104
Positive	5	9	18	14	7	5	53
Do.....per cent..	6.3	64.3	41.2	52.0	50.0	83.3	50.9

ANOPHELES RECONNAISSANCE

TECHNIC

The collection and handling of mosquito larvæ offered a considerable problem for solution. The dippers used were 4 inches deep and had a spout on one side guarded with fine-mesh screening so that excess water could be poured out without loss of larvæ. These dippers had a ferrule into which was inserted a 3-foot walking cane as a handle. These dippers proved to be highly satisfactory for the collection of Philippine anophelines. Collections were made by forcing the dipper down into the water near collections of bamboo roots, grass, etc., and allowing the water to run into the dipper from among the roots or grass. It was frequently found that larvæ were hidden so far up among the roots of bamboo that several dips in the same place were necessary to dislodge them.

Larvæ so collected were removed with a medicine dropper, the tip of which had been enlarged, and placed in a half pint fruit jar half filled with water from the place in which the larvæ were collected. These jars were sealed by the use of a jar rubber and cap held down by the ordinary device used on fruit jars. The number and exact location were noted on a label pasted on the jar and an elaborated descriptive note entered in the collection book.

The collection jars were placed in special holding boxes and transported until the end of the day. At each prolonged stop the jars were opened for the ingress of fresh air. At night the collections were carefully inspected; all fourth-stage larvæ were removed and placed in hot water momentarily to kill and straighten them out. They were then preserved in formalin-filled shell vials, numbered and brought to Manila for identification. All

larvæ smaller than fourth stage were fed a mixture of two parts of powdered litmus milk and one part of powdered dried blood serum until they were large enough to remove. Some specimens were allowed to breed out and the adult mosquitoes identified. We have transported live larvæ for periods up to twenty-one days. It was found that the larvæ breed out much more slowly than in nature. By aërating the water at frequent intervals and subjecting the containers to as little agitation as possible, it was found that the loss of larvæ from death was very small.

CLASSIFICATION

There has been considerable confusion regarding the specific identity of Philippine *Anopheles* but the history of this question cannot be considered in this report. The more recent papers bearing on this subject are by Manalang,(40) Baisas,(41) and King.(42, 43, 44) The three articles by King are particularly important in this regard. Based on a study of the above sources and others, and in consultation with W. V. King, we have used the following classification of Philippine *Anopheles:*

1. *Anopheles aitkeni* James, 1913.
2. *Anopheles barbirostris* Van der Wulp, 1884.
3. *Anopheles filipinæ* Manalang, 1929.
4. *Anopheles fuliginosus* Giles, 1900.
5. *Anopheles gigas* var. *formosus* Ludlow, 1909.
6. *Anopheles hyrcanus* var. *nigerrimus* Giles, 1900.
7. *Anopheles hyrcanus* var. *sinensis* Wiedemann, 1928.
8. *Anopheles insulaeflorum* Swellengrebel and Swellengrebel de Graaf, 1920 (*A. aitkeni* var.).
9. *Anopheles karwari* James, 1903.
10. *Anopheles kochi* Donitz, 1901.
11. *Anopheles leucosphyrus* Donitz, 1901.
12. *Anopheles lindesayi* var. *benguetensis* King, 1931.
13. *Anopheles litoralis* King, 1932. (Salt-water *ludlowi.*)
14. *Anopheles ludlowi* Theobald, 1903. (Fresh-water *ludlowi.* In the past *litoralis* has also been included as *A. ludlowi.*)
15. *Anopheles maculatus* Theobald, 1901.
16. *Anopheles mangyanus* Banks, 1906. (Probably identified in previous collections as *A. minimus*, possibly *A. filipinæ* and perhaps *A. funestus* of Manalang.)
17. *Anopheles minimus* Theobald, 1901. (*A. febrifer* Banks, 1914, included chiefly *A. mangyanus*, probably, but also included *A. minimus* and *A. filipinæ*. See King.(44) Manalang's *A. funestus* is chiefly *A. minimus* but may include sometimes *A. mangyanus*. It may be necessary to call the Philippine *minimus, Anopheles minimus* var. *flavirostris* Ludlow, 1914.)
18. *Anopheles parangensis* Ludlow, 1914.
19. *Anopheles philippinensis* Ludlow, 1902.

20. *Anopheles pseudobarbirostris* Ludlow, 1902.
21. *Anopheles subpictus* var. *indefinitus* Ludlow, 1904. ("A. rossi.")
22. *Anopheles tesselatus* Theobald, 1901.
23. *Anopheles umbrosus* Theobald, 1903.
24. *Anopheles vagus* var. *limosus* King, 1932.

It must be borne in mind that this list is not a final one by any means. Most of our collections have lot numbers and have been deposited in the Bureau of Science collection, Manila, or in the collection of W. V. King. Therefore, they will be available for reclassification later if necessary.

In Table 13 the catches of *Anopheles* larvæ are listed by species, and by province and town or barrio. The numbers used correspond to the serial numbers in the above classification list. No. 17 is *A. minimus* and it will be noted that this species was found in nearly all parts of the Archipelago.

In Table 14 are listed the species encountered on this survey and the times each was collected. It will be seen that no specimens of *A. leucosphyrus* or *A. parangensis* were caught. The species most frequently taken were *A. minimus*, *A. barbirostris*, and *A. maculatus* in the order named.

Certain species were at times taken together in the same breeding place. Some of these associations are shown in Table 15. *Anopheles minimus* with *A. barbirostris*, and *A. minimus* with *A. maculatus* were the commonest associations.

BIOLOGICAL CONSIDERATION

In general, our observations as to the habits of Philippine *Anopheles* are in line with those of Barber et al.,(1) Tiedeman,(9) Mieldazis,(12) Manalang,(46, 47, 48, 49) and King.(42, 43, 44)

BREEDING PLACES

In Table 16 are listed the total number of breeding places from which *Anopheles* larvæ were taken. In Table 17 these collections are further classified.

It will be noted that a fairly wide variety of breeding places were sampled. Streams predominated, then rivers and irrigation ditches in the order named. Four collections were made in wells, *A. minimus* being taken twice in this location. In regard to *A. minimus* breeding in wells, see Russell and Santiago.(50)

In Table 18 are listed the breeding places of *A. minimus* and *A. mangyanus*. Where *A. mangyanus* was found it was usually taken in association with *A. minimus* but the reverse is seldom

true. Not until the reconnaissance was almost over was the work of King(44) available.

We had, of course, noticed some variation in the *minimus* larvæ but until King(44) made the significance of this variation clear, we had followed the usual plan of calling these variants *A. minimus*. When it became apparent that *A. mangyanus* is entitled to specific status, King began a reparation of our *minimus* collection into the two groups, *mangyanus* and *minimus*. In Table 19 there is a list of breeding places where *A. mangyanus* was found. In Table 20 is a list showing the reverse, *A. mangyanus* but not *A. minimus*.

It is clear from Tables 13 to 20 that one should not be too dogmatic in describing the breeding places of the *A. minimus* group. In any part of the Archipelago, clear, shady streams are most apt to harbor larvæ of this group. Frequently, if bamboo is growing along the stream bank the larvæ are most likely to be found breeding among its roots, but *A. minimus* is a versatile creature and does not limit itself to the classical breeding places.

The fact that our collections could not be standardized as to time and area makes it unwise to draw any but general conclusions as to prevalence and breeding habits of the various species. It can be noted, however, that latitude and longitude per se seem to have very little influence within this Archipelago on the prevalence and breeding habits of *Anopheles* larvæ. We could see no essential differences, for example, in the habits of *A. minimus* in Ilocos Norte and Balabac, in Cagayan and Davao, in Bohol and Jolo.

MALARIA TRANSMISSION

No infected mosquitoes were caught during this survey. Epidemiological evidence in the table below points strongly at only *A. barbirostris* and the *minimus* group. They were usually abundant in malarious places and were uncommon or absent in nonmalarial spots.

With very few exceptions these species, and particularly *A. minimus* and *A. mangyanus*, were always associated with malaria. One notable exception was South Ubian Island, in Sulu Province. Here, with a spleen index of 28.2 per cent and a clear-cut history of malaria, we were unable to find *Anopheles* larvæ. There is no fresh water on the island except in water jars, and we failed to find *Anopheles* in these or in salt-water pools. The explanation seemed to be clear, however, for the Moros of this island are constantly going and coming from Tawitawi, some

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species.*

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
ABRA		1932			
Abra River	34	Jan. 6	4, 14, 17, 19, 24	R U C	446
Bangued	31	do	2, 3, 17, 21	S G C B	In town.
Do	33	do	2, 3, 17	S G C	447.3
Palao	32	do	2, 3, 17	R U C	Sinalang River.
ALBAY					
Bogtong	27A	Mar. 1	2, 3, 16, 17, 24	S G C	
Daraga	27	Feb. 29	16, 17	S G C	
Guinobatan	24	do	3, 16, 17	S G C B	
Do	25	do	2, 3, 16, 17	R G B C	
Legaspi	21	do	2, 3, 17	S G B C	
Do	22	do	21	P U C	
Do	23	do	2, 3, 17	S G M	
Mauraro	26	do	2, 16, 17	S G C	
ANTIQUE					
Pandan	8	Feb. 23	2, 17	S G C B	West.
Do	9	do	2, 17	S G C	Near foothills.
Do	10	do	17	S G C B	East.
Do	11	do	2, 20	D U	Shaded.
Do	12	do	2, 17	S G C B	
BATAAN		1930			
Abucay	8	Nov. 5	7	I U M	Town.
Do	9	do	2, 17	R G C	Do.
		1931			
Do	1	Nov. 9	17	S C U	Hacienda Natib.
Do	2	Nov. 10	16, 17	S C U	Do.
Do	3	do	2, 17	S G C	Do.
Do	4	do	7, 17	I U C	Do.

Do	5	do	7, 20	S C U	Do.
Do	6	Nov. 11	16, 17	S G C	Do.
Do	7	do	7, 17	S U C	Do.
Do	8	do	16, 17	S G C B	Do.
Do	9	do	2, 17	S G C B	Do.
Do	10	do	7, 17, 20	S U C	Do.
Do	11	do	16, 17	S G C B	Do.
Do	12	do	16, 17	S G C B	Do.
Do	13	Nov. 13	7, 16, 17	S G C B	Do.
Bagac					Town. No larvæ found.
Bagac to Balanga	8–14B	July, 1931	3, 4, 8, 14, 15, 17, 21	S G C	Trail 9 collections.
Balanga	11	Nov., 1930	4	P C U	Town.
		1931			
Balanga	1	Nov. 21	15, 17	S G C	Hacienda Mataan.
Do	2	do	17	S G C B	Do.
Do	3	do	3, 17	S G C	Do.
Do	4	do	15, 17	S G C B	Do.
Do	5	do	2, 15, 17	I G C	Do.

* The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
BATAAN—continued		1931			
Balanga	6	Nov. 21	17	S G C	Hacienda Mataan.
Do	7	do	17	S G C	Do.
Do	8	do	17	S G C	Do.
Do	9	Nov. 23	3, 15, 17	S G C	Do.
Do	10	do	3, 15, 17	S G C	Do.
Do	11	do	15, 17	S G C B	Do.
Do	12	do	17	S G C B	Do.
Do	13	Nov. 24	17	S G C	Do.
Do	14	do	17	S G C B	Do.
Do	15	do	17	S G C B	Do.
Do	16	do	3, 17	S G C	Do.
Do	17	do	17	S G C	Do.
Do	18	do	3, 17	S G C	Do.
Do	19	do	17	S G C	Do.
Do	20	do	17	S G C B	Do.
Do	21	Nov. 25	17	S G C B	Do.
Do	22	do	17	S G C B	Do.
Do	23	do	15, 17	S G C	Do.
Do	24	do	17	S G C	Do.
Do	25	do	15, 17	S G C B	Do.
Do	26	Nov. 26	15, 17	I G C	Do.
Do	27	do	3, 17	I G C	Do.
Do	28	do	15, 17	I U C	Do.
Do	29	do	15, 17	R U C	Do.
		1930			
Cupang	12	Nov. 5	2, 17	I U C	Balanga barrio.
Do	13	do	4, 7	P C U	Do.

Corregidor		June, 1931			No *Anopheles* larvæ found. Entire island searched.
		1930			
Dinalupihan	19	Nov. 3	2, 17	R U C B	Layac.
Hermosa	1	Nov. 4	2, 17	S G C	Town.
Do	3	do	7	P C U	Do.
Limay	18	Nov. 6	2, 15, 17	R G C B	Do.
Limay-Bagac	1-7B	July, 1931	2, 3, 8, 16, 17	S G C	Trail 14 collections.
Mariveles		June, 1931	17	S G C	
		1930			
Orani	4	Nov. 4	4, 7	P U M	Town.
Do	20	Nov. 13	21	O P U	Do.
Do	21	do	21	O P U	Do.
Do	22	do	21	O P U	Do.
Do	23	do	21	O P U	Do.
Orion	14	Nov. 5	2, 4	P U C	
Do	15	do	21	P U C	
Do	16	do	2, 4, 17, 19, 21	I U C	

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *pawangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
BATAAN—continued		1930			
Orion	17	Nov. 6	21	P U C	
Samal	5	Nov. 5	21	I U C	
Do	6	do	21	P U C	
Do	7	do	7	S C U	
		1931			
Sisiman	1	May 22	2, 17	S G C	
BATANGAS					
Batangas	15	Dec. 17	2, 14, 15, 24	R P U	
Cuenca	19	Dec. 18	2, 15	S G C	
Ibaan	12	Dec. 17	14, 15, 17	R C U	
Rosario	13	do	2, 17	S G C	
Do	14	do	15, 17	S G M	
Sabang	11	do	2, 14, 15, 17	S G C	
Tuy	16	do	2, 3, 15, 16, 17	S G C B	
Do	17	do	2, 15, 17	S G C B	
Do	18	do	2, 17	S G C B	
BOHOL		1932			
Agricultural Colony	5	Mar. 29	2, 17	R U C	
Balilihan	1A	do		S G C	No larvæ found.
Calape	3	do	15, 17	S G C B	
Carmen	4	do	2, 17	S G C B	
Loon	2	do	2, 15, 17	S G C	
Tagbilaran	1	do		O R U	Do.
BULACAN		1930			
Baisa	237	Dec. 22	2, 4, 19, 21, 24	P U C	
Malolos					
Marilao					

Tungcong Manga	2	Jan. 6	2, 8, 15, 16	S G C	Matuid Creek.
Do	3	do	4, 19	P G C	
Do	7	Jan. 24	2, 3, 14, 21, 24	R U C	Lower Alat.
Do	8	do	3, 7, 15, 17, 24	S G C	
CAGAYAN		1932			
Aparri		Mar. 13		O U	No larvæ found.
Gattaran	12	do	2, 17	S G C	
Iguig	11	do	17	S G M	
Do		do		R U C	No larvæ in river 3 km south of Iguig.
Tuguegarao	14	do	17	R U C B	
CAMARINES SUR					
Dayandan	34	Mar. 2	2, 7, 17	S P G C	
Naga	34A	do	7, 17	P G	
Pinit, San Jose Road	35	do	3, 17	S G C	Km 31.
Do	36	do	2, 3, 17	S G C	
Do	37	do	2, 3, 16, 17	S G C	
Pasacao	38	do	2, 3, 7, 15, 16, 17	S G C B	

* The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
CAPIZ		1932			
Buntog	16A	Apr. 8		R M U	No larvæ found.
Capiz	15	do	21	O P U	
Do	16	do	7, 19, 21	P U C	
New Washington	13	Feb. 24	13, 21, 23	O P U	
Do	14	do	7, 19, 21	P U	
CAVITE					
Indang	2	Mar. 22	15, 17	S G C	
Do	2A	do	2, 15, 17	S G C	
Naic	8	do	17	S G M	
Ternate	1	do	17	W U C	
CEBU		1930			
Cebu	238	Nov. 29	13, 21	O P U	
Labangon	239	do	14, 17	S G C	
		1932			
Mambaling	1	Jan. 17	2, 17	S G C B	
Mandawe	2	do	13	O P U	Km 7 from Cebu.
Do	3	do	13	O P U	Do.
Tabunoc	3A	do	14, 15, 17	S G C	
Toledo	6	Mar. 30	2, 17	S G C	Km 3.5 from Toledo toward Cebu.
DAVAO		1930			
Davao	4	Jan. 30	2, 20, 22	S G M	
Matina	6	do	17	S G M	
Mintol	5	do	17	I M U	
Do	10	Jan. 31	17, 20, 22	I M U	
Do	11	do	2, 17, 22	S G M	
Pakiputan	8	do	2, 23	R U M	
Do	8A	do	22	P G	

Do	9	do	2, 20, 24	P G	
Talomo	7	Jan. 30	17	S G C	
ILOCOS NORTE		**1932**			
Bangui	1	Jan. 3	2, 3, 7, 15, 17, 22	I U C	K 603
Do	2	do	2, 4, 7, 19, 24	I U C	K 601
Do	3	do	2, 7	I U C	599
Do	4	do	2, 17	I U C	597
S. Bautista	5	do	2, 17	S G C	595-596
Km 591	6	do	2, 4, 24	R G C	591
Km 588	7	do	2, 3, 4, 7, 19	I G C	588
Baruyan River	8	do	2, 15, 17	R U C	
Asido	9	do	2, 17	S G C	575+
Kms 566-65	10	do	2, 3, 7, 17	S G C	
San Mateo	11	Jan. 4	2, 17	R U C	
Laoag	12	do	2, 14, 17, 19	R G C B	Laoag River.
Bacarra	13	do	17	I U C	
Laoag	14	do	17	S G C	536
Do	15	do	17	I U C	535

[A] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.

D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.

G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
ILOCOS NORTE—continued		1932			
Badoc	16	Jan. 4	2, 3, 4, 17	S G C	Town.
Do	17	do	2, 4, 17	S G M	495
Paoay	19	do	2, 7, 20	R G M	508
Batac	21	do	2, 17	S G C B	516
Do	22	do	17	S G C	518
Do	22A	do	2, 3, 17	S G C B	526.67
ILOCOS SUR					
Bantay	27	Jan. 5		S G C B	442. None found.
Do	28	do		S G M B	445. None found.
Do	29	do	2, 17, 20	S G M	447.29
Cabugao	23	do	2, 3, 17	S G C B	475.8
Do	24	do	4, 14, 21	R U C	
Do	25	do	17	S G C B	East.
Suyo	35	Jan. 6	2, 17	R U C	
Do	36	do	2, 3, 15	P C U	
Do	37	do	17	S G C	
Do	38	do	2, 17	S G C B	
Vigan	26	Jan. 5	2, 3, 17, 19, 21	S U M	Southeast of Vigan.
Do	30	do	2, 17, 21	S U M	Half a km north.
ILOILO					
Iloilo	21	Apr. 4	12	O P U	Fishpond.
Leon	19	do		S G M B	Foothill. None found.
Do	19A	do	14, 24	R G C	River East.
Passi	17	do	2	R U C	Main river.
Do	18	do	2, 21	S G C	Branch of river.
Jaro	20	do	2	R U C	River.

ISABELA					
Echague	10	Mar. 12	2, 7, 17	S G C B	
Ilagan		Mar. 14			None found in river. All small streams dry.
LAGUNA		1931			
Calauan	10B	Dec. 16	2, 17	S G C	Macalauan stream.
Do	10C	do	2, 16, 17, 21, 24	S G C	Mabacan branch.
Do	10D	do	2, 4, 7, 17, 21, 24	S G C	Flat stream.
Do	10E	do	2, 4, 5, 7, 19, 20, 21	P C G	Tangke spring.
Mabitac	7	Dec. 15	2, 9, 17	S G C B	One km north.
Magdalena	10	Dec. 16	2, 17	S G C B	East.
Do	10A	do	2, 16, 17	S G C B	Do.
Santa Maria	8	Dec. 15	2	S G C	West.
Do	8A	Dec. 26	2, 10, 17	S G C B	Do.
Do	8B	do	2, 10, 17	S G C B	South.
Do	8C	do	2, 4, 17	S G C B	Do.
Do	8D	do	24	P U	East.
Siniloan	5	Dec. 15	2	S G C	
Do	6	do	2, 6, 7, 20	D U	

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni*.
2. *barbirostris*.
3. *filipinæ*.
4. *fuliginosus*.
5. *gigas* var. *formosus*.
6. *hyrcanus* var. *nigerrimus*.
7. *hyrcanus* var. *sinensis*.
8. *insulaeflorum*.

Anopheles—
9. *karwari*.
10. *kochi*.
11. *leucosphyrus*.
12. *lindesayi* var. *benguetensis*.
13. *litoralis*.
14. *ludlowi* (fresh-water).
15. *maculatus*.
16. *mangyanus*.

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis*.
19. *philippinensis*.
20. *pseudobarbirostris*.
21. *subpictus* var. *indefinitus*.
22. *tessellatus*.
23. *umbrosus*.
24. *vagus* var. *limosus*.

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.

D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.

G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
LAGUNA—continued		1931			
Dayap to Pila	5	Dec. 30	2, 16, 17	R G C B	Bridge, 81.86
Do	6	do	2, 17	P U	Bridge, 83.80
Do	7	do	2, 3, 17	P U B	Bridge, 83.84
Do	8	do	2, 17	I U C	Bridge, 83.84
Do	9	do	2, 17	I U C	Bridge, 83.86
Do	10	do	17	S G M	Between 83.84 and 83.93
Do	11	do	2, 17	S G M	Bridge 83.93
Do	12	do	2	R G M B	Bridge 85.62
		1932			
Pila to Santa Cruz	13	Jan. 6	2, 17	S G M	Bridge 87.29
Do	13B	do	17	P U C	Bridge 87.29
Do	14	do	2, 6, 17	I M U	Bridge 87.76
Do	15	do	2, 17	S G C	Bridge 90+
Do	16	do	2, 10, 17, 20	I C U	Between 90+ and 90.33
Do	17	do	2, 6, 17	I C U	Bridge 90.33
Do	18	do	2, 6, 7	I C U	Bridge 93.35
Do	19	do	6	P U	Bridge 95.96
Do	20	do	6, 22	I M U	Bridge 95.96
Do	21	do	6, 17, 22, 24	I M U	Bridge 96.22
Do	22	do	2, 4, 6, 7, 17, 24	R M G B	Bridge 96.90
Santa Cruz to Magdalena	23	Jan. 13	2, 17	R G M	Bridge 98.89
Do	24	do	2, 3, 17	S G C	Bridge 100
Do	25	do	2, 17	S G C B	Bridge 100.49
Do	26	do	2, 17	S G C	Km 120
Do	27	do	2, 17	S G C	Bridge 117.43
Do	28	do	17	S G C	Bridge 117.21
Calauan to San Pablo	29	Jan. 20	2, 3, 15, 17	R G C	Road bridge 85.2
Do	30	do	2, 15, 17	S G C	Bridge 86.1

Do	31	do	15, 17	R G C	Bridge 89.73
Do	32	do	2, 17	R G C	Malinao River.
San Pablo to Rizal	33	Jan. 27	2, 15, 17	S G C	Between Km 91 and 92
Do	34	do	15, 17	S G C	Bridge 93.67
Do	35	do	15, 17	S G C	Bridge 95.02
Do	36	do	17	S G C	Bridge 95.08
Do	37	do	2, 24	I U M	Bridge 98.14
Rizal to Nagcarlan	38	Feb. 3	10, 15, 17	S G C	Km 99
Do	39	do	15, 17	S G C	Bridge 100.44
Do	39A	do	2, 20	P U	
Do	40	do	15, 17	I U C	Bridge 102.59
Do	41	do	2, 3, 7, 17	I G C	Bridge 102.95
Nagcarlan to Lilio	42	Feb. 10	17	S G C	103.43
Do	43	do	17	R C G	San Diego River.
Do	44	do	2, 17	S G C B	106.21
Do	45	do	17	S G C B	106+
Do	46	do	17	S G C B	106.44
Do	47	do	2, 17	S G C B	107.42

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tessellatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
LAGUNA—continued		1932			
Nagcarlan to Lilio	48	Feb. 10	2, 17	S G C	107.68
Lilio to Magdalena	49	Feb. 17	2, 15, 17	S G C B	108.21
Do	50	do	15, 17, 20	S G C B	109.33
Do	51	do	17	I U	110.87
Do	52	do	2, 17	I U	110.87
Do	53	do	17	I U	111.20
Do	54	do	2, 17	S U	112+
Do	55	do	17	S G C	112.95
Do	56	do	2, 15, 17	I U	Km 114–115
Calauan to Bay	57	Feb. 24	2, 7, 17, 24	S U M	Km 75.1
Do	58	do	2, 4, 6, 7, 17, 24	I C U	Km 73.
Do	59	do	2, 3, 7, 17, 22	S U C	Km 74.4
Do	60	do	2, 7, 17, 20	I G C	Km 72
Do	61	do	2, 17	R U C	Km 72.8
Do	62	do	2, 17	R G C	Km 71.17
Do	63	do	2, 17	I U C	Km 71.17
Bay to Los Baños	64	Mar. 9	17	I G C	Km 69
Do	65	do	2, 4, 20	R M U	Km 71.15
Do	66	do	2, 4, 20, 21	I U M	Km 70.92
Do	67	do	2, 17	R G C	Km 70.57
Do	68	do	2, 17	S G C	Km 70.5
Do	69	do	17, 24	S G C	Km 67
Los Baños to Calamba	70	do	17, 24	S G M B	Km 65.38
Do	71	do	3	I U C	Km 65.07
Do	72	do	2, 24	P U	Km 65.07
Do	73	do	2, 3	P G	Km 63.54
Do	74	do	17	I G C	Km 63.23
Do	75	do	24	P G	Km 62.85
Do	76	do	24	P U	Km 62.85

Do	77	do	3, 17	S G C	Km 59.89
Do	78	do	21	L U C	Pansol beach.
Do	79	do	3	L U C	Do.
Do	80	do	21, 24	P U M	Pansol Railroad station.
Do	81	do	3, 17	R U C	Km 58.80
Do	82	do	4, 6	I M U	55
Calamba to Cabuyao	83	Mar. 10	21	R U C	West of Calamba School.
Do	84	do	4, 21	R U C	54.31
Do	85	do	24	I M U	52.53
Do	86	do	17	I M U	49.90
Do	87	do	2, 17	S G C B	47.95
Do	88	do	2, 17, 21	R G C B	47.06
Do	89	do	2, 3, 17	R G M	45.39
Do	90	do	24	F C U	45.06
Cabuyao to Santa Rosa	91	do	4, 6, 17, 21	I U M	45.06
Do	92	do	17	S G C	43.75
Do	93	do	17	I G M	43.38
Do	94	do	2, 4, 17	I G M	42.90

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—

1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—

9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—

17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tessellatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 18.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
LAGUNA—continued		1932			
Cabuyao to Santa Rosa	95	Mar. 10	2, 6, 17, 24	I G M	42.41
Do	96	do	2, 3, 15, 17, 24	R G C	42.20
Santa Rosa to Biñan	97	Mar. 12	6	I U C	40.20
Do	98	do	17	S M G B	39.74
Do	99	do	6, 17, 24	S M G	39.25
Do	100	do	17	S M G	39.18
Do	101	do	2, 4, 17, 21	R M G	36.55
Biñan to San Pedro	102	do	2, 17	R U C	Near public market.
Do	103	do	2, 17, 21	R G C B	31.44
Do	104	do	2, 3, 17	S G C B	31.2
Sambat to Majayjay	105	Mar. 16	2	I G C	113.79
Do	106	do	2, 17	I U C	114.24
Do	107	do	2, 17	I G C	114.24
Do	108	do	17	S G M	115.15
Do	109	do	2, 10, 17	I G C	Between 116–117
Do	110	do	17	I U C	Do.
Do	111	do	17	I G C	118.75
Do	112	do	2, 17	I U C	118.75
Do	113	do	17	S G M	118.75
Do	114	do	2, 17	S G M	119.85
Do	115	do	2, 3, 17	S G C	120.22
Do	116	do	2, 3, 17	S G C	120.50
Do	117	do	15, 17	I G C	120.60
Pagsanjan to Cavinti	118	do	17, 24	R G C	101.42 clear, shady, grassy.
Do	119	do	2	R G C	Near market, clear shady, grassy.
Do	120	do	15, 17	S G C	Between 104–105 clear, shady, shallow.
Do	121	do	2, 19, 20, 24	S G U	Between 107–108 clear, shallow, open.
Do	122	do	2, 3, 17	S G M B	Between 108–109 muddy bamboo.
Do	123	do	2	P G M	Near 110, muddy, vegetated, shady debris.

Do	124	do	2, 3, 17, 24	S M G	110+ muddy, shady, roots grasses.
Do	125	do	2	S M G	110+ muddy, shallow, shady.
Do	126	do	2, 17	R G M	111 muddy debris.
Linga to Pinagbayanan	127	do	21	L U C	Linga Beach, nipa, grasses.
Do	128	do	17	R G M B	Pinagbayanan River, lilies, muddy, bamboo.
San Pablo and Alaminos	129	do	2, 15, 17	S G C	87.74 clear, shady.
Do	130	do	2, 15, 17	S G C	86.14 clear, shady.
Do	131	do	15, 17	S G C	Southeast of Alaminos School.
San Pablo and Tiaong	132	do	15, 17	S G C	91.64
Do	133	do	17	S G C	92.06
Do	134	do	17	S G C	93.87
Do	135	do	2, 15, 17	S G C	96.22
Do	136	do	2, 3, 17, 24	S G C	98.73
Do	137	do	24	P U M	101+
Do	138	do	17	R G C	101+
Santa Cruz Beach	139	do	4, 7, 19, 21	L U C	Santa Cruz Beach.
Calamba to Santo Tomas	140	do	2	S G C B	62.84
Do	141	do	15, 17, 24	S G C	65.41

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulæflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *longirostris.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus*
22. *tessellatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
LANAO		1930			
Kolambugan	1	Dec. 23–26	2, 20, 23, 24	Swamp U	Salt-water swamp.
Do	2	do	15	S G C	Clear, moderately shaded.
Do	3	do	2, 7, 23, 24	R U C	Not shaded.
LA UNION		1932			
Bauang	39	Jan. 7	2, 17	S G C B	Shady bamboo.
Burgos	40	do	2, 3, 15, 17	R U C	309
Saitan	41	do	2, 3, 15, 17	S G C B	246
LEYTE					
Carigara	18	Feb. 26	2, 17	R G C	Rapid, clear.
Talibong (Biliran Island)	19	do	15, 17, 24	S G C	
MINDORO					
Camboag (San Jose)	6	Feb. 22	2, 3, 7, 17	S G C	Deep, moderately shaded.
Do	7	do	2, 17	S G C	
Caminawit	1	do	21	P C	
Pandurucan	2	do	13	O P U	
Do	3	do	21, 24	P G C	
San Jose	4	do	17	S G M	
Do	5	do	2, 17	S G M	
MANILA		1930			
Malacañang	18–19	Feb. 6	2, 6, 7, 24	P C U	
		1932			
Malate	1	Mar. 19		O P U	No larvæ found.
Maypajo	2	do	13	O P U	
MASBATE					
Masbate	15	Feb. 25	15	I G C	
Do	17	do	15, 16, 17	S G C	
Tagba	16	do	2, 3, 15, 17	S G C	

MISAMIS					
Cagayan	1	Nov. 30	3, 17, 19	S G C	
Gingoog	2	Dec. 3-9	3, 4, 15, 17, 20	R G C B	
Gusa	3	Nov. 30	17	S G C	
MOUNTAIN					
Baguio	104	May 2	5, 12, 15	I U C	Pack and Kisad Roads.
Do	105	May 4	7, 15	F U C	K 276
Do	105A	do	4, 15	S G C	K 277
Do	106	May 5	5, 12, 15	I U C	Bureau of Forestry.
Do	107	do	5, 12, 15	F U C	Pack Road.
Do	108	May 8	5, 12	S G C	Camp John Hay.
Do	95	Apr. 29	5, 12	S G C	Do.
Do	96	May 30	7, 5, 12	S G C	Do.
Do	94	May 28	7, 15	I U C	Trinidad Valley.
Cervantes		Jan., 1931	15, 17	S G C	
Kiangan		Dec., 1931		S U C	No larvæ found.
Loo		do		R U C	Do.
Tawan		do		S G C	Do.

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulæflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tessellatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.

D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.

G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
NUEVA ECIJA		1932			
Bongabon	2	Mar. 10	2, 3, 4, 17	S G C B	
Cabiao	13	Mar. 15	2, 17, 20	R G C B	
Cabu	1	Mar. 10	2, 17	S G M	
Puncan	4	do	2, 17	S G C	
San Jose	3	do	2, 17	S G C B	
NUEVA VIZCAYA					
Bagabag	7	Mar. 11	2, 3, 17	S U M	
Bambang	6	do	2, 3, 17	S U M	
Bayombong	8	do	2, 14, 17	R U C	
Rosario (Km post 298)	9	Mar. 12	2, 17	S U C B	
Santa Fe	5	Mar. 11	2, 15, 17, 24	S G C	
OCCIDENTAL NEGROS					
Bacolod	9	Mar. 31	2, 7, 17, 21	S G C B	K 3–5 north on road to Talisay.
Do	10	do	2, 17	S G C B	K 2–5 Talisay.
Isabela	11	Apr. 1	2, 17	S G C B	Southeast.
Murcia	12	Apr. 2	7, 24	W M U	K 2 north.
Do	13	do	24	S G M	50 yd. north.
Do	14	do	2, 17, 21, 24	R G C	Sumag River.
San Carlos	7	Mar. 30	15, 17	S G C B	K 2 southwest.
Do	8	do	21, 24	S U M	K 1–5 northwest.
ORIENTAL NEGROS					
Dumaguete	29	Feb. 12	15, 17	S G C B	Km 3 south.
Do	30	do	17, 20	I G C	Km 5 south.
PALAWAN					
Balabac	5	Apr. 3	2, 17	S G C	
Baja Point	6	Apr. 5	2, 17	S G C	Malabuniga River.
Coron		Mar. 28			Streams dry; none found.

Culion	1	do	2, 17	S G C	1 km east of hospital.
Cuyo		Mar. 29			None found.
Iwahig	2	Mar. 30	17, 21	S U C	Balsahang River.
Do	3	Mar. 31	17	S G C	Lakondola River.
Do	4	Apr. 1	7, 17	S G C	Kasuyan River.
Puerto Princesa		Mar. 30			None found.
PAMPANGA		1930			
Del Carmen		Jan. 6	2, 7, 17, 19	R U C B	
Do		Jan. 23	2, 17	R U C	
Do		Mar. 18	2, 17	R U C	
Do		Feb. 6	2, 17	R U C B	
Floridablanca		Feb. 19	14, 17, 21, 24	R G C	
Porac	34	do	4, 14, 17, 21	R G C B	
PANGASINAN		1932			
Alaminos	45	Jan. 8	2, 17, 21	S G B	1.5 km southeast
Infanta	47	do	19	P	Town.
Lingayen	44	do	21	P O	Do.

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *fornosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *bengalensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
PANGASINAN—continued		1932			
Mabini	46	Jan. 8	17	R G B	0.3 km east.
Malasiqui	43	Jan. 7	17	S G M	0.1 km northeast.
Urdaneta	42	do	2, 17, 20	R P M	202.5
RIZAL		1930			
Antipolo-Las Piñas	47	Mar. 5	14	P U C	
		1931			
Las Piñas	225	Nov. 13	13, 21	O P U	
		1932			
Navotas	4	Mar. 19			None found.
Parañaque		do	13	O P U	
		1930			
Pasay	26	Feb. 13	13, 21	O P U	
San Francisco del Monte	1	Jan. 4	2, 17	S G C B	
San Juan del Monte	5	Feb. 7	2, 6, 7	R M U	
SAMAR		1932			
Allen	20	Feb. 27	2, 15	R P U C	
Do	20A	do	2, 15 16, 17	R U C	
SORSOGON					
Bulusan	28	Mar. 1	15, 17	S G C	
Putiao	33	do	2, 3, 15, 16, 17	S G M	
Rizal Barrio	31	do	2, 3, 15, 16, 17	I U C	
Do	32	do	2, 17	W S C	
Sorsogon	29	do	2, 3, 15, 16, 17	S G C	
Do	30	do	2, 15, 16, 21, 24	P U C	

SULU					
Astorias, Jolo	23	Feb. 5	2, 17	S G C	
Bato-bato		do	17	S G C	
Bongau		do	17	S G C	
Jolo, Jolo	21	do	17	S G M	Camp Romandies.
Do	22	do	21	O P U	Camp Listt.
Do	24	do	17	S U C	
Do	26	do	17, 20, 21	S U C	
Do	25A	do	17	S G C	
Lapac Island	27B	Feb 7			No larvæ found.
Lugus Island	23	do	21	L U C	
Do	28A	do	21	L U C	
Do	28B	do	21	W G C	
Maimbong, Jolo	18A	Feb. 5	17	S G C	
Manubul Island	27C	Feb. 7			No fresh water on island. No larvæ found.
Pantao, Jolo	20A	Feb. 5	17	S G C	
Sisai Island	26	do	17, 20, 21, 24	S G C	
Do	27	Feb. 6	17, 20	S G C	

* The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *giyas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water).
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tesselatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
ZAMBALES—continued		1931			
Castillejos	17A	July 30	14, 16, 17	S G B C	Aninguay River.
Do	17B	....do....	2, 17, 24	S G B C	Do.
		1932			
Iba	49	Jan. 8	2, 17	S G M	Town.
		1931			
Olongapo	14	July 29	1, 2, 16, 17	R G C	Batoc River.
Do	14B	....do....	2	F U	Near Batoc River.
Olongapo-Dinalupihan	13F	Aug. 1	16, 17	S G C	Beyond Santa Rita.
Do	13G	....do....	2, 15, 16, 17	S G C	Mountain stream.
Do	13H	....do....	16, 17	S G C	Do.
Do	13I	....do....	17	S G C	Do.
Do	13K	....do....	17	S G C	Do.
San Antonio	20	July 30	4	I M C	North.
Do	21A	....do....	4, 14, 17, 19	S C G	Calpalman.
		1932			
San Marcelino	50	Jan. 9	4, 17	S G C	Foothills north.
		1931			
San Narciso	44B	July 30	7, 21	S C U	Kayaran stream.
Santa Rita	13A	July 29	2, 16, 19, 21	S C G	Near cabaret.
Do	13B	....do....	14, 15, 16, 17, 20	S C G	Near school.
Subic area	2	July 28	14, 15	S C U	Southwest.
Do	3	....do....	14, 15	S C U	Do.
Do	3A	....do....	14, 15	S C G	Do.
Do	4B	....do....	14, 15, 17	S C G	Do.
Do	4C	....do....	4, 14, 19, 21	S C G	Do.
Do	5B	....do....	2	S C G	Do.
Do	9A	....do....	14, 15, 16, 17	S C U	Do.

Do	9C	do	16, 17	S C U	Do.
Do	10	do	15, 17	R C G	Do.
Do	12A	July 29	1, 16, 17	S C G	Northeast.
Do	12B	do	15, 17	S C G	Do.
Do	15A	July 30	1, 2, 14, 15, 16, 17	S C G	Do.
ZAMBOANGA					
Kabasalan	1B	Sept. 27	2	S U C	
Do	1C	do	8	S U C	
Do	1D	do	2, 8	S U C	
Do	101	do	1, 8, 17	S G C	
Do	102	do	8, 17	S G C	
Do	103	do	2, 17	S U C	
Do	104	do	2	S G C	
Do	105	Sept. 29	2, 17, 22	I C U	
Do	106	do	17, 22	I C U	
Do	107	Sept. 28	1, 8	S G C	

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni.*
2. *barbirostris.*
3. *filipinæ.*
4. *fuliginosus.*
5. *gigas* var. *formosus.*
6. *hyrcanus* var. *nigerrimus.*
7. *hyrcanus* var. *sinensis.*
8. *insulaeflorum.*

Anopheles—
9. *karwari.*
10. *kochi.*
11. *leucosphyrus.*
12. *lindesayi* var. *benguetensis.*
13. *litoralis.*
14. *ludlowi* (fresh-water)
15. *maculatus.*
16. *mangyanus.*

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis.*
19. *philippinensis.*
20. *pseudobarbirostris.*
21. *subpictus* var. *indefinitus.*
22. *tessellatus.*
23. *umbrosus.*
24. *vagus* var. *limosus.*

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.

D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.

G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 13.—*Anopheles larvæ taken during the reconnaissance, listed by province, town or barrio, and species*—Continued.

Locality.	Lot No.	Date.	Larvæ.[a]	Breeding places.[a]	Remarks.
ZAMBOANGA—continued		1931			
Kabasalan	108	Sept. 28	2	S G C	
Do	109	do	22	I U C	
Do	110	do	17	I U C	
Do	111	Sept. 29	2	I U C	
Do	112	Sept. 30	17	S G C	
Do	112A	do	3, 17	S G C	
Do	113	Oct. 1	17	S G C	
Do	114	Oct. 2	17	S G C	
Do	114A	do	2, 17	S G C	
Do	115	do	2, 8, 17, 20	S G C	
Do	116	do	17	S G C	
Do	117	do	2, 8, 17, 24	S C U	
Do	118	Oct. 3	2, 8, 17	S C U	
Do	118A	do	2, 8	S C U	
Do	119	do	2, 6, 20	I U C	
Do	120	do	2, 15, 17	I U C	
Do	121	Oct. 5	2, 17, 22	I U C	
Do	121	do	2, 7, 15, 22	S G C	
Do	122	do	8	S G C	
Do	123	Oct. 6	20, 22	P U	
Do	124	do	2, 15, 24	P U	
Do	125	Oct. 6	2	R U C	
Do	126	do	17, 19, 22	S G C	
Do	127	do	17	S G C	

		1932			
Limajon	12	Feb. 2	17	S G C	
Mercedes	15	Feb. 3	2, 17	S G C	
Tulungatung	16	do	17	S G C	100 yds. from town.
Do	17	do	2	S G C	0.5 km from town.
Tumaga	18	Feb. 2	17	S G C B	
Zamboanga	14	Feb. 3	2, 17, 21	I C U	

[a] The species of the mosquito larvæ collected are indicated by numbers and the types of breeding places by letters, as follows:

Anopheles—
1. *aitkeni*.
2. *barbirostris*.
3. *filipinæ*.
4. *fuliginosus*.
5. *gigas* var. *formosus*.
6. *hyrcanus* var. *nigerrimus*.
7. *hyrcanus* var. *sinensis*.
8. *insulæflorum*.

Anopheles—
9. *karwari*.
10. *kochi*.
11. *leucosphyrus*.
12. *lindesayi* var. *benguetensis*.
13. *litoralis*.
14. *ludlowi* (fresh-water)
15. *maculatus*.
16. *mangyanus*.

Anopheles—
17. *minimus* (*flavirostris*).
18. *parangensis*.
19. *philippinensis*.
20. *pseudobarbirostris*.
21. *subpictus* var. *indefinitus*.
22. *tesselatus*.
23. *umbrosus*.
24. *vagus* var. *limosus*.

BREEDING PLACES

S, stream.
R, river.
P, pool.
L, lake.
F, rice field.
D, ditch, still.
I, irrigation ditch, flowing.
W, well.
O, ocean salt water.
B, bamboo.
G, shaded.
U, unshaded.
C, clear.
M, muddy.

TABLE 14.—*Species of Anopheles encountered in survey and number of times found.*

Serial No.	Species.	Times collected.
1	*Anopheles aitkeni*	5
2	*Anopheles barbirostris*	254
3	*Anopheles filipinæ*	68
4	*Anopheles fuliginosus*	35
5	*Anopheles gigas* var. *formosæ*	7
6	*Anopheles hyrcanus* var. *nigerrimus*	17
7	*Anopheles hyrcanus* var. *sinensis*	48
8	*Anopheles insulæflorum*	14
9	*Anopheles karwari*	2
10	*Anopheles kochi*	6
11	*Anopheles leucosphyrus*	0
12	*Anopheles lindesayi* var. *benguetensis*	6
13	*Anopheles litoralis*	10
14	*Anopheles ludlowi*	25
15	*Anopheles maculatus*	109
16	*Anopheles mangyanus*	50
17	*Anopheles minimus*	378
18	*Anopheles parangensis*	0
19	*Anopheles philippinensis*	21
20	*Anopheles pseudobarbirostris*	30
21	*Anopheles subpictus* var. *indefinitus*	61
22	*Anopheles tesselatus*	15
23	*Anopheles umbrosus*	4
24	*Anopheles vagus* var. *limosus*	53

TABLE 15.—*Association of two or more species of Anopheles in the same breeding place.*

Serial Nos.	Species in association.	Times found together.
2 and 17	*Anopheles barbirostris* and *A. minimus*	178
15 and 17	*Anopheles maculatus* and *A. minimus*	101
16 and 17	*Anopheles mangyanus* and *A. minimus*	26
2, 15, and 17	*Anopheles barbirostris*, *A. maculatus*, and *A. minimus*	36
3 and 17	*Anopheles filipinæ* and *A. minimus*	61
3, 15, and 17	*Anopheles filipinæ*, *A. maculatus*, and *A. minimus*	17
2, 3, and 17	*Anopheles barbirostris*, *A. filipinæ*, and *A. minimus*	44
17 and 24	*Anopheles minimus* and *A. vagus* var. *limosus*	21
2, 17, and 24	*Anopheles barbirostris*, *A. minimus* and *A. vagus*	16
2, 17, and 21	*Anopheles barbirostris*, *A. minimus*, and *A. subpictus*	13

seven miles west, where they secure their drinking water. Whole families go to this island sometimes to spend one or more days. Tawitawi is known to be highly malarious. Furthermore, these people are fishermen and go to the neighboring islands to spend weeks at a time. Therefore, they have had many

opportunities to become infected in places where *A. minimus* is breeding.

The only other notable exception to the rule of an association between malaria and the *A. minimus* group was at Davao where, at Mintol, we found a splenic index of only 9.7 per cent among Japanese school children who were living on an estate through which runs a stream breeding *A. minimus* larvæ. Due to the economic depression, Paris-green treatment of this stream had been discontinued for some months and larvæ were abundant. The low splenic index could be accounted for by the fact that these children and all laborers on this estate were compelled to use bed nets. There are no exceptions to this rule, and rigid supervision is carried out.

Elsewhere, as noted in the tables, malaria and the *A. minimus* group were always closely associated, but the mere fact of asso-

TABLE 16.—*Breeding places in which collections were made.*

Breeding place.	Times visited.
Streams	318
Rivers	77
Irrigation ditches	72
Pools, fresh water	40
Pools, salt water	22
Lakes	6
Rice fields	4
Wells	4
Ditch, still, unshaded	2
Swamp	1
Total collections	546

TABLE 17.—*Classification of breeding places.*

Breeding place.	Times visited.
Stream:	
Clear water	266
Muddy water	44
Shaded water	174
Unshaded water	38
River	77
Pool, fresh water	39
Lake	6
Rice field	4
Irrigation ditch	72
Well	4
Salt-water pool or pond	22
Other places:	
Ditch, unshaded	2
Swamp	1

TABLE 18.—*Breeding places of A. minimus and/or A. mangyanus.*

Breeding place.	Collections of *Anopheles minimus* and/or *A. mangyanus*.
Stream:	
Clear water	225
Muddy water	37
Shaded water	141
Unshaded water	23
Bamboo at edge	72
River:	
Clear water	47
Muddy water	7
Shaded water	32
Unshaded water	23
Bamboo at edge	17
Irrigation ditch:	
Clear water	36
Muddy water	9
Shaded water	14
Unshaded water	35
Other places:	
Pool—	
Stream, shaded, clear	2
Stream, unshaded, muddy	1
Shaded	2
Unshaded	1
Unshaded, bamboo roots	1
Well—	
Shaded, clear	1
Unshaded, clear	1

ciation with malaria is obviously not enough to prove that a mosquito is a malaria carrier. It must be remembered that in order to be a factor in the transmission of human malaria in any given region a species of *Anopheles* must obviously have certain characteristics. It must be distributed in sufficient numbers close enough to habitations. It must desire human in preference to animal blood and strongly enough to enter houses for meals. Finally, it must be susceptible to infection. Not a great deal is known about the interplay of these factors in the life history of Philippine anophelines.

The first requirement of density in numbers and proximity to habitations is fulfilled by a majority of the Philippine *Anopheles*. As to the second factor, very little information is available. Only the work of Laurel(51, 52) has been published. This observer found that thirty-one out of thirty-nine blood meals of

A. minimus (*"funestus"*) reacted for human and none for animal tests. The reverse was true of eight other species, including *A. barbirostris*. Of the latter, thirteen were tested; seven reacted to cow sera, none to human.

Anopheles minimus adults are rarely taken inside human habitations in the daytime, as noted by Russell,(53, 54) but large numbers may be trapped if a sleeping human is used for bait. See Manalang.(49, 55) Numbers have been caught in bed nets. See Barber et al.(1)

As to the final test; namely, the susceptibility to infection, a great deal more information is required. The first work to determine susceptibility was done by Banks,(56) who claimed to have experimentally infected *Myzomyia ludlowi*. Since his mosquitoes came from salt water they were probably of the species that King has named *A. litoralis* and which is so-called in our classification. This work was inconclusive for, as Manalang(45) points out, Banks may have been mistaken in his diagnosis of the sporozoites.

TABLE 19.—*Breeding places where larvæ of A. minimus but not A. mangyanus were taken.*

Breeding place.	Collections of *Anopheles minimus*.
Stream:	
Clear water	48
Muddy water	5
Shaded water	46
Unshaded water	4
Bamboo	15
River:	
Clear water	6
Muddy water	2
Shaded water	8
Unshaded water	1
Bamboo at edge	3
Irrigation ditch:	
Clear water	12
Muddy water	
Shaded water	2
Unshaded water	10
Pool:	
Stream, shaded, clear	1
Stream, unshaded, muddy	
Shaded	
Unshaded	3
Unshaded, bamboo	

TABLE 20.—*Breeding places where larvæ of A. mangyanus but not A. minimus were taken.*

Breeding place.	Collections of *Anopheles mangyanus.*
Stream:	
Clear water	13
Muddy water	1
Shaded water	13
Unshaded water	1
Bamboo	3
River:	
Clear water	4
Muddy water	1
Shaded water	3
Unshaded water	2
Bamboo at edge	2
Irrigation ditch:	
Clear water	1
Muddy water	
Shaded water	
Unshaded water	1
Pool:	
Stream, shaded, clear	1
Stream, unshaded, muddy	
Shaded	
Unshaded	1
Unshaded, bamboo	

The next studies of experimental infection were made by Walker and Barber.(57) Table 21 includes only strictly comparative figures and not the totals. Both gut and gland infections are included.

TABLE 21.—*Walker and Barber's summary of experimental malaria infections in Philippine Anopheles.*

Species.	Insects dissected.	Infected.	
		Number.	Per cent.
Anopheles febrifer [a]	162	108	66.7
Anopheles maculatus	3	2	66.7
Anopheles rossi [b]	187	35	18.7
Anopheles barbirostris	100	6	6.0
Anopheles sinensis	12	0	0.0

[a] *Anopheles febrifer* included what in our report is *A. mangyanus*, also *A. minimus* and perhaps *A. filipinæ*.

[b] *Anopheles rossi* probably included *A. litoralis*, *A. subpictus* v. *indefinitus*, *A. vagus*. *A. sinensis* is *A. hyrcanus* v. *sinensis*.

The remaining published evidence is that of Manalang.(45, 46, 47, 55, 58, 59) This observer in thousands of dissections of several Philippine species, including *A. barbirostris, A. fuliginosus, A. hyrcanus* var. *sinensis, A. karwari, A. maculatus, A. minimus* (*"funestus"*), *A. philippinensis, A. subpictus* var. *indefinitus* (*"rossi"*), *A. tesselatus,* and *A. vagus* has only found *A. minimus* infected. (Whether the infected individuals did or did not include *A. mangyanus* is uncertain.) On one occasion Manalang(60) found a wild-caught *A. maculatus* infected but this was after artificial incubation in a laboratory cage. On another occasion one heavily infected stomach was found in *A. vagus.*(55)

Therefore, all available evidence, at the present time, points to the *minimus* group as the chief vectors of malaria in the Philippines, but it would indeed be rash to assume that only this group is ever guilty in this Archipelago.

There is no evidence in our reconnaissance that salt-water *Anopheles* play any part in malaria transmission in the Philippines. Epidemiologically, our results tend to incriminate the *minimus* group above all others, but our tables do not rule out the distinct possibility that other species may occasionally be transmitting malaria.

SUMMARY AND CONCLUSIONS

This paper reports a malaria reconnaissance in two hundred fourteen towns or barrios in forty of the forty-eight provinces of the Philippine Islands. Five hundred forty-six separate collections of *Anopheles* larvæ were made, in which were found twenty-two of the twenty-four known Philippine species. These collections are analyzed as to type of breeding place, and frequency and associations of species. A discussion of the classification and biology of Philippine *Anopheles* is presented.

During this reconnaissance 1,237 blood-smear examinations were made. These are reported in the tables together with 7,869 spleen palpations. A discussion of spleen palpation in general and its application to malariometry in the Philippines is given.

There is also included a general discussion of climate and altitude in relation to malaria in the Philippines with appropriate maps and notes. As a result of our observations we conclude that:

1. Malaria is a serious and widespread disease in the Philippines, being prevalent from Ilocos Norte to Tawitawi and from Balabac to Samar.

2. This disease is not universally or uniformly present in the Archipelago. It is not prevalent along the low coastal plains, on the flat plateaus, or in the mountains above 2,000 feet. It is mildly endemic in some places, severely so in others, and hyperendemic in still others.

3. Malaria and the *minimus* group of *Anopheles* are almost always directly associated in the Philippines. This observation, taken together with published facts as to the prevalence of infected *minimus* mosquitoes both in nature and in laboratory experiments, leads us to suspect strongly that the chief vectors of malaria here are in the *minimus* group of *Anopheles*.

4. The *minimus* group of *Anopheles* breeds chiefly in streams, rivers, and irrigation ditches, more in shady than open places and in clear than muddy water. It distinctly prefers clean, fresh, flowing water. But this group is not restricted to these habitats. We have found it occasionally in still pools and in wells. We have never found it in salt water, in rice fields, or in any water at a higher altitude than 2,000 feet.

5. In the light of the above conclusions, it is not surprising that we also conclude that malaria in the Philippines is primarily a disease of foothills. It is found chiefly in two zones; namely, that between the coastal plain and higher ground and that between this higher ground and the mountains.

6. Altitudes of less than 2,000 feet, per se, have no marked influence on the prevalence of malaria in the Philippines. It is change of contour rather than altitude which directly affects malaria prevalence here. Above 2,000 feet malaria transmission apparently does not occur. Our evidence in this respect, although entirely consistent, is not yet great enough to permit a dogmatic statement.

7. With due regard to occasional cases of chicken pox, measles, and schistosomiasis, we conclude that spleen palpations give as good an index in the Philippines, as elsewhere, of the prevalence of malaria.

REFERENCES

1. Barber, M. A., Alfonso Raquel, Ariston Gusman, and A. P. Rosa. Malaria in the Philippine Islands, II. The distributions of the commoner anophelines and the distributions of malaria. Philip. Journ. Sci., § B 10 (1915) 177–247.
2. Nichols, H. J. Medical survey of the town of Taytay, XIII. Filariasis, malaria, etc. Philip. Journ. Sci. § B 4 (1909) 280.
3. Musgrave, W. E., et al. Sanitary survey of the San Jose Estate and adjacent properties on Mindoro Island, with special reference to the epidemiology of malaria. Philip. Journ. Sci. § B 9 (1914) 137–197.

4. PADUA, R. G. Findings on the malaria investigations within the four selected zones in Laguna Province. P. I. Health Service Monthly Bull. 3 (1923) 47–66.
5. PADUA, R. G. Findings on malaria investigations in the Iwahig Penal Colony. P. I. Health Service Monthly Bull. 3 (1923) 249–265.
6. PADUA, R. G. Incidence of splenic enlargements in a malarious district of the Philippines. Journ. P. I. Med. Assn. 3 (1923) 1–6.
7. MANALANG, C. Malaria inquiry and control in Mindanao and Sulu. P. I. Health Service Monthly Bull. 7 (1927) 281–298.
8. MANALANG, C. Malaria control in the Philippines. Far Eastern Association of Tropical Medicine. Trans. 8th Congress 2 (1927) 705–11.
9. TIEDEMAN, W. v. D. Malaria in the Philippines. Journ. Prev. Med. 1 (1927) 205–254.
10. EJERCITO, A. Malaria surveys and controls in Mindanao and Sulu. P. I. Health Service Monthly Bull. 8 (1928) 537–88.
11. EJERCITO, A. Summarized report on malaria surveys and control in Mindanao and Sulu. P. I. Health Service Monthly Bull. 9 (1929) 277–83.
12. MIELDAZIS, J. J. Preferential breeding conditions of Anopheles in the Philippine Islands. Philip. Journ. Sci. 41 (1930) 59–63.
13. EJERCITO, A., and A. G. LAUREL. Report on the malaria survey of the proposed negative barrio at Malaking Patag (Cogonal Grande), Culion Island, P. I. Monthly Bull., Philippine Health Service 11 (1931) 467–479.
14. HOLT, R. C., and P. F. RUSSELL. Spleen survey of the eastern shore of Bataan Province, Luzon. Philip. Journ. Sci. 45 (1931) 211–219.
15. LULL, G. C. Since the days of the Empire. Milit. Surgeon 69 (1931) 512–517.
16. ASH, JAMES E. Quarterly Report of the U. S. Army Medical Department Research Board (July 20, 1922). (Not published.)
17. SIMMONS, JAMES S., and JOE H. ST. JOHN. The prevalence and distribution of malaria on the Island of Corregidor, Philippine Islands. Milit. Surgeon 64 (1929) 710.
18. SIMMONS, JAMES S., JOE H. ST. JOHN, F. H. K. REYNOLDS. A malaria survey at Fort Stotsenburg, P. I. Milit. Surgeon 67 (1930) 1.
19. CRAIG, C. F. The Malarial Fevers. Wm. Wood and Co., New York (1909) 11 and 393.
20. BEYER, HENRY OTLEY. Various publications.
21. KROEBER, A. L. Peoples of the Philippines—Anthropological Handbook Fund. Lancaster Press, Lancaster, Pa. (1928).
22. Census of the Philippine Islands 2 (1918).
23. SYDENHAM, The works of. Latham's translation of Greenhill's Latin edition made for the Sydenham Society, London 1 (1848) Medical Observations, Chapter 5.
24. BAKER, W. E., T. E. DEMPSTER, and H. YULE. Report of a committee assembled to report on the causes of the unhealthiness which has existed at Kurnaul, etc. (1847). Reprinted in Records Malaria Survey India 1 No. 2: 1–62.
25. CHRISTOPHERS, S. R. Trans. Fourth Congress Far Eastern Assoc. of Trop. Med. 1 (1921).

26. MANSON, Sir PATRICK, quoted by Hehir. Malaria in India. Oxford Univ. Press, London (1927) 411.
27. Proc. Third All-India Sanitary Conference, Lucknow (Jan. 19–27, 1914). Supplement to Ind. Journ. Med. Res. 4 (1914) 15.
28. BOYD, M. F. An Introduction to Malariology. Harvard University Press, Cambridge (1930) 147–174.
29. TUBANGUI, M. A. Personal communication. Also a paper read before the Philippine Society of Parasitology (April 15, 1932).
30. DARLING, S. T. The spleen index in malaria. So. Med. Journ. 17 (1924) 590–596.
31. CHRISTOPHERS, S. R., and KHAZAN CHAND. Measurement in centimeters of the enlarged spleen in children and its correction for size of child by a factor based on anthropometric measurement. Ind. Journ. Med. Res. 11 (1924) 1065–1080.
32. OUDENDAL, A. J. F. Transactions Sixth Congress Far Eastern Association Tropical Medicine 2 (1925) 235.
33. DARLING, S. T. Splenic enlargement as a measure of malaria. Ann. Clin. Med. 4 No. 9 (1926) 695–712.
34. BEAN, R. B., and W. BAKER. Some racial characteristics of the spleen weight in man. Am. Journ. Physical Anthropology 2 (1919).
35. Treatment of Malaria. U. S. Public Health Reports 34, No. 52 (Dec. 26, 1919).
36. COLLINS, R. K. An experience with intensive quinine treatment under field conditions. So. Med. Journ. 19 No. 5, (1926).
37. RUSSELL, P. F. Reports of the Straits Settlements rural sanitation campaign, Penang (1927). (Unpublished.)
38. MACDONALD, G. The significance of the various degrees of splenic enlargement in malarious communities. Rec. Malaria Surv. of India 2 (1931) 569–602.
39. STEPHENS, J. W. W., and S. R. CHRISTOPHERS. Reports to the Malaria Committee of the Royal Society, VI Series (1902) 23.
40. MANALANG, C. Morphology and classification of the Philippine varieties of Anopheles aconitus Donitz, 1902, and Anopheles minimus Theobald, 1901. Philip. Journ. Sci. 43 (1930) 247–262.
41. BAISAS, F. E. The barbirostris-hyrcanus group of the Philippine Anopheles. Philip. Journ. Sci. 44 (1931) 425–448.
42. KING, W. V. The Philippine varieties of Anopheles gigas and Anopheles lindesayi. Philip. Journ. Sci. 46 (1931) 751–757.
43. KING, W. V. The Philippine Anopheles of the rossi-ludlowi group. Philip. Journ. Sci. 47 (1932) 305–342.
44. KING, W. V. Three Philippine Anopheles of the funestus-minimus group. Philip. Journ. Sci. 48 (August, 1932). (In press.)
45. MANALANG, C. Notes on malaria transmission. Philip. Journ. Sci. 37 (1928) 123–129.
46. MANALANG, C. Malaria transmission in the Philippines, II. Infection of Anopheles funestus Giles (minimus) with notes on its density, probable range of flight, and larva control. Philip. Journ. Sci. 45 (1931) 368–381.
47. MANALANG, C. Malaria transmission in the Philippines, III. Density and infective density of Anopheles funestus Giles. Philip. Journ. Sci. 46 (1931) 47–59.

48. MANALANG, C. Malaria transmission in the Philippines, IV. Meteorological factors. Philip. Journ. Sci. 46 (1931) 247–255.
49. MANALANG, C. Malaria transmission in the Philippines, VI. The dark-night factor. Philip. Journ. Sci. 46 (1931) 371–375.
50. RUSSELL, P. F., and DOMINGO SANTIAGO. Anopheles minimus larvæ from wells in Laguna Province, P. I. Philip. Journ. Sci. (In press.)
51. LAUREL, A. G. Identification of ingested blood in the stomachs of anopheline mosquitoes by means of the precipitin test. P. I. Health Service Monthly Bull. 10 (1930) 153–166.
52. LAUREL, A. G. Precipitin test on anopheline blood meal. P. I. Health Service Monthly Bull. 10 (1930) 537–538.
53. RUSSELL, P. F. Daytime resting places of Anopheles mosquitoes in the Philippines, first report. Philip. Journ. Sci. 46 (1931) 639–649.
54. RUSSELL, P. F. Daytime resting places of Anopheles mosquitoes in the Philippines, second report. Am. Journ. Trop. Med. (In press.)
55. MANALANG, C. Malaria transmission in the Philippines, I. The natural vector. Philip. Journ. Sci. 45 (1931) 241–250.
56. BANKS, C. S. Experiments in malarial transmission by means of Myzomyia ludlowi Theobald. Philip. Journ. Sci. § B 2 (1907) 513–535.
57. WALKER, E. L., and M. A. BARBER. Experiments on the transmission of malaria. Philip. Journ. Sci. 9 (1914) 381–439.
58. MANALANG, C. Summary report for 1928 of the central laboratory, division of malaria control. P. I. Health Service Monthly Bull. 5 (1929) 14–16.
59. MANALANG, C. Does the amount of malaria depend on the numbers of transmitting mosquitoes? Journ. Trop. Med. and Hyg. 34 (1931).
60. MANALANG, C. Personal communication to P. F. R. (1931).

271104——5

ILLUSTRATIONS

PLATE 1

Climate map of the Philippine Islands, showing the seasons and the rainfall.

PLATE 2

Temperature map of the Philippine Islands.

PLATE 3

Map of the Philippine Islands, showing places visited during a malaria reconnaissance and the endemicity based on spleen index.

PLATE 4

Typical *Anopheles minimus* group breeding stream in Jolo, Sulu Province, showing partial shade and rapid flow of water; also the type of collecting dipper.

PLATE 5

An *Anopheles minimus* group breeding place along the bank of Cabiao River, Laguna Province, Luzon, showing partial shade, bamboo, and water hyacinth.

PLATE 6

FIG. 1. Santa Fe, Nueva Vizcaya Province, Luzon; altitude, 1,900 feet. Malaria is hyperendemic in this town. Note the stream.

2. The stream at Santa Fe immediately to the left of the area shown in the foreground of fig. 1. A breeding place of mosquitoes of the *Anopheles minimus* group.

PLATE 7

FIG. 1. A view, toward the north, from Puncan Barrio, Nueva Ecija Province, Luzon. The flat ricefield country stretching south from the edge shown in this photograph is not malarious, and does not breed *Anopheles minimus;* but Puncan is directly in the transition zone between this flat country and the mountains and is highly malarious. Its streams breed mosquitoes of the *A. minimus* group.

2. Coast line at Pasacao, Camarines Sur Province, Luzon. Note the proximity of foothills. This town is malarious.

3. Pathfinder Estate, Kabasalan, Zamboanga Province, Mindanao. The streams and ditches on this estate are breeders of the *A. minimus* group; and the estate, situated in the transition zone between coastal mangrove swamps and mountains, is malarious.

TEXT FIGURE

FIG. 1. Diagram of spleen sizes according to Boyd.

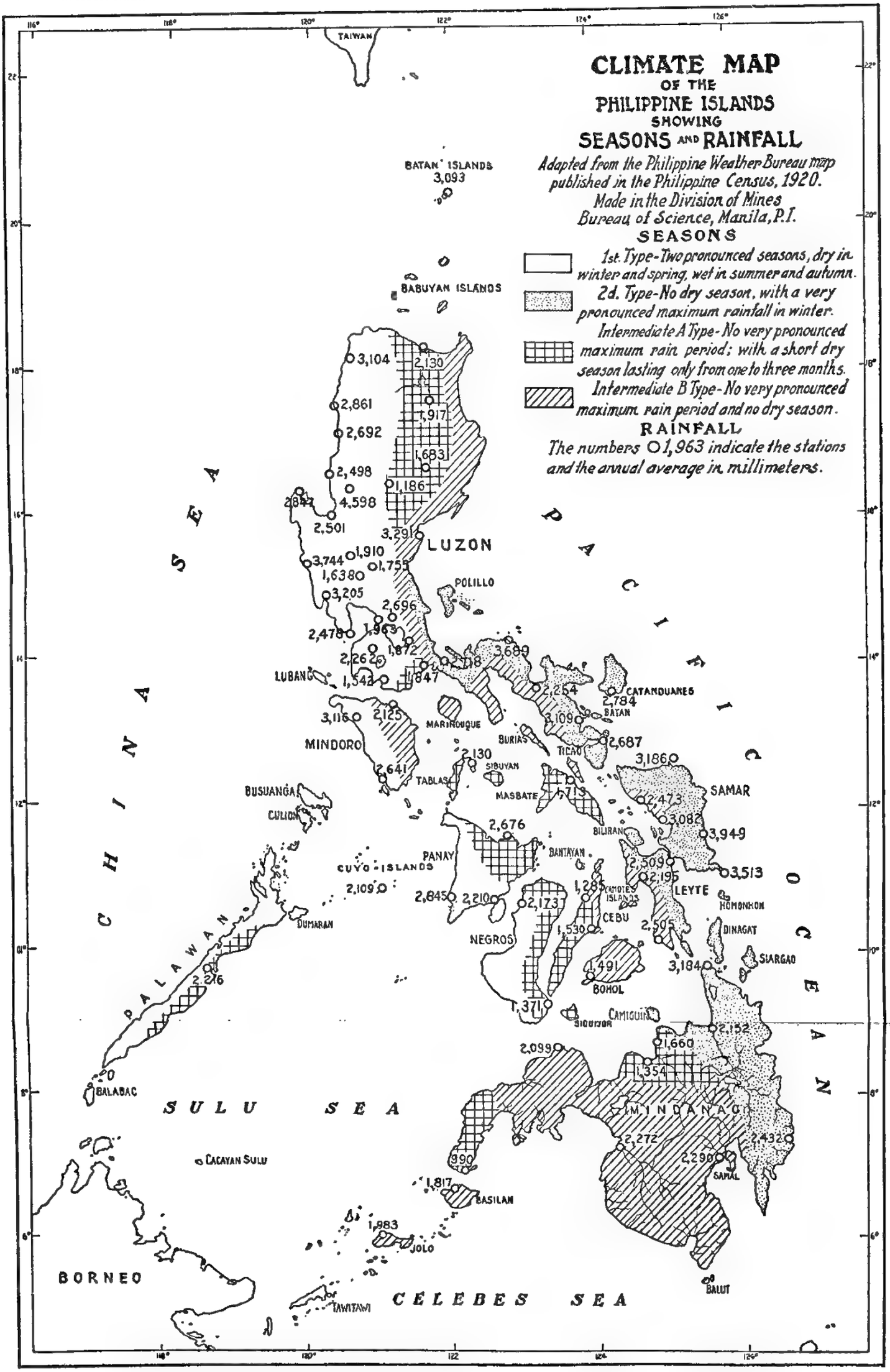


PLATE 1. CLIMATE MAP OF THE PHILIPPINE ISLANDS, SHOWING THE SEASONS AND THE RAINFALL.

CLIMATE MAP
OF THE
PHILIPPINE ISLANDS
SHOWING
SEASONS AND RAINFALL

Adapted from the Philippine Weather Bureau map published in the Philippine Census, 1920.
Made in the Division of Mines
Bureau of Science, Manila, P.I.

SEASONS

1st. Type-Two pronounced seasons, dry in winter and spring, wet in summer and autumn.

2d. Type-No dry season, with a very pronounced maximum rainfall in winter.

Intermediate A Type-No very pronounced maximum rain period; with a short dry season lasting only from one to three months.

Intermediate B Type-No very pronounced maximum rain period and no dry season.

RAINFALL

The numbers ○1,963 indicate the stations and the annual average in millimeters.

PLATE 1. CLIMATE MAP OF THE PHILIPPINE ISLANDS, SHOWING THE SEASONS AND THE RAINFALL.

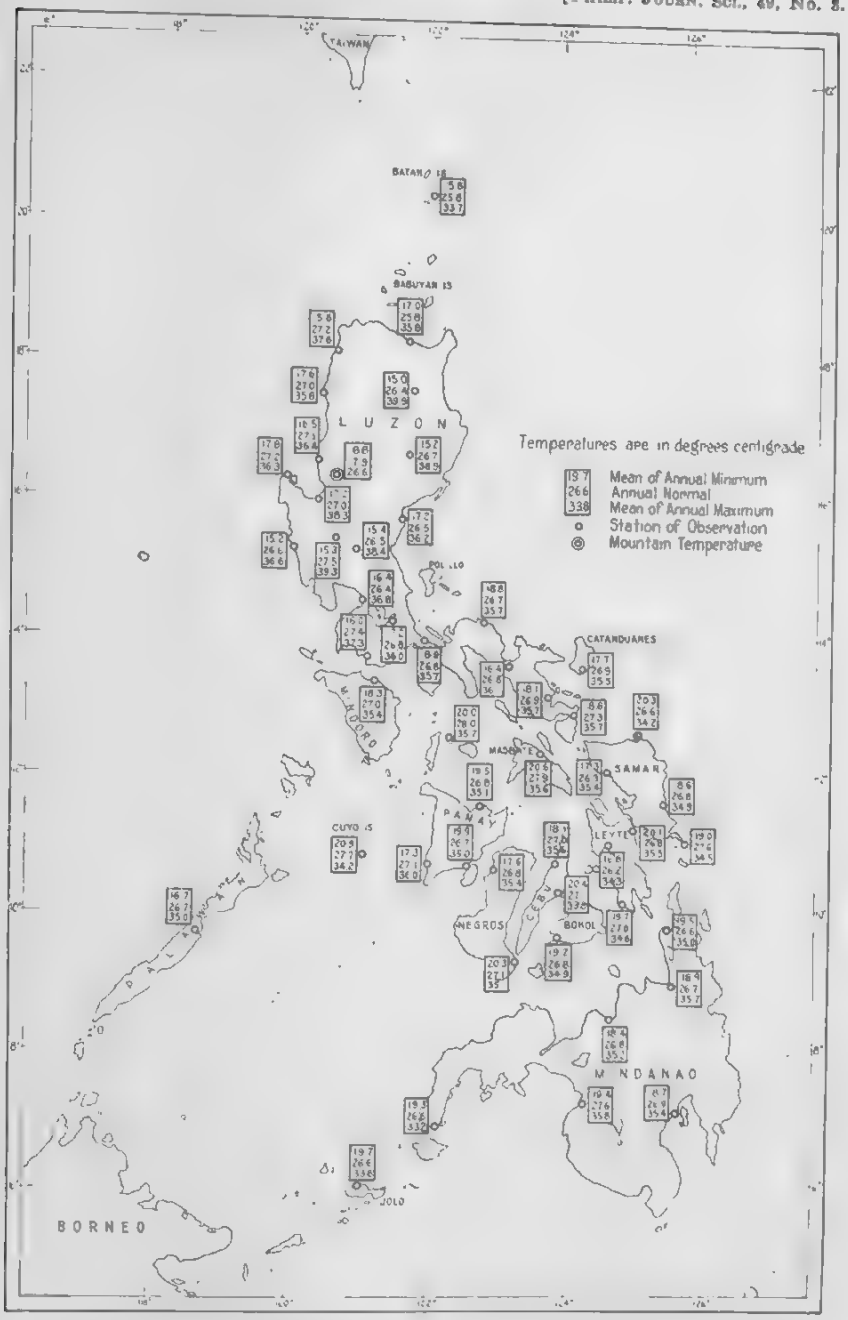


PLATE 2. TEMPERATURE MAP OF THE PHILIPPINE ISLANDS.

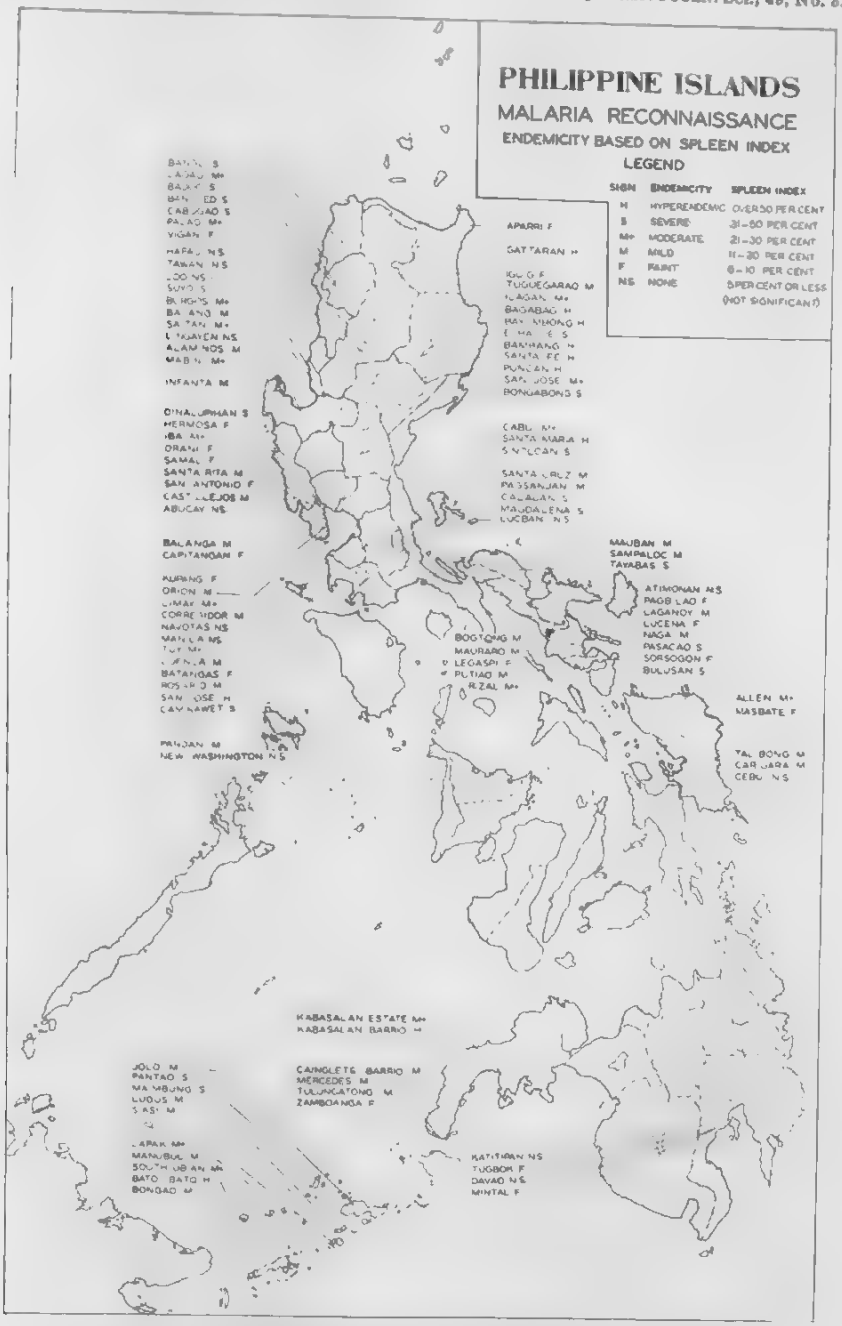
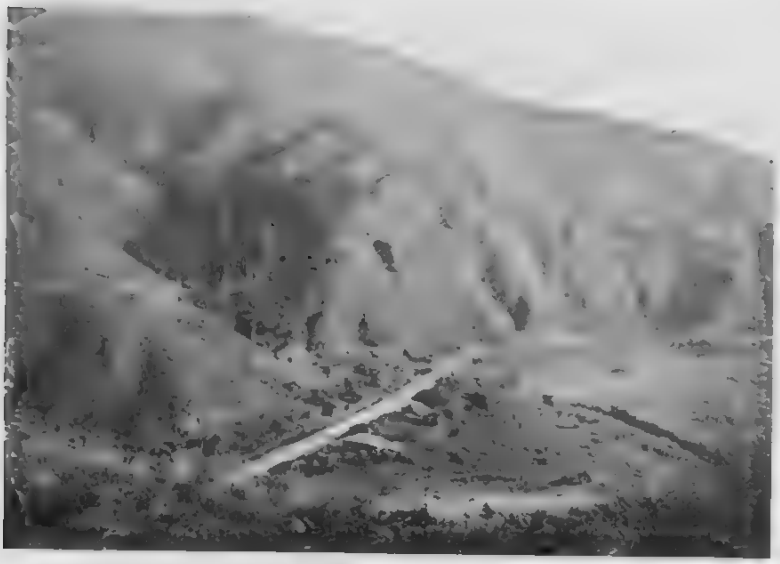


PLATE 3. THE PHILIPPINE ISLANDS, SHOWING PLACES VISITED DURING A MALARIA RECONNAISSANCE AND THE ENDEMICITY BASED ON SPLEEN INDEX.

PLATE 4.

PLATE 5.



1

2

PLATE 6.

1

2

3

PLATE 7.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM EASTERN ASIA (DIPTERA), XI[1]

By CHARLES P. ALEXANDER

Of Amherst, Massachusetts

THREE PLATES

The very interesting crane flies discussed at this time were all taken at altitudes of between 3,500 and 11,000 feet on Mount Omei, Szechwan, western China, by the Reverend Mr. Franck. The genera and subgenera *Trichotipula, Macgregoromyia, Adelphomyia, Phyllolabis, Rhabdomastix, Gnophomyia,* and *Teleneura* are herewith added to the Chinese list. I wish to express my very deep thanks to Mr. Franck and Mr. Parish for the privilege of studying this exceptional series of Tipulidæ. All types are preserved in my collection.

TIPULINÆ

TIPULA (TRICHOTIPULA) POLYTRICHA sp. nov. Plate 1, fig. 1; Plate 2, figs. 23, 24, 25, 26.

General coloration of mesonotum black; thoracic pleura chiefly black; antennæ black, the basal two segments yellow; knobs of halteres yellow at apices; legs chiefly black; wings cream-colored, chiefly concealed by extensive brown seams on cord and other veins, and by darkened central areas in the primary basal cells; wing membrane with abundant macrotrichia in all cells almost to arculus; wing veins with a virtually complete series of trichia on all veins and crossveins; male hypopygium with the ninth tergite tridentate at tip; basistyle and tergite partly fused with the sternite.

Male.—Length, about 11 millimeters; wing, 12.3.

Frontal prolongation of head short and stout, testaceous brown; nasus short but distinct, blackened; palpi black. Antennæ (male) of moderate length, if bent backward extending about to root of halteres; scape and pedicel light yellow; flagellum black; basal enlargement of segments relatively small and little differentiated; verticils shorter than the segments; terminal

[1] Contribution from the entomological laboratory, Massachusetts State College.

segment long-conical, about one-third as long as the twelfth. Front and anterior vertex yellow, the posterior portion of head dark brown, with abundant black setæ; vertical tubercle inconspicuous or lacking.

Pronotum brown. Mesonotum shiny black. Pleura brownish black, variegated with paler brown on the sutures, dorsopleural region, and meron; sternopleurite with setæ. Halteres brown, the apices of the knobs conspicuously light yellow. Legs with the coxæ brownish yellow, the posterior coxæ paler; trochanters obscure yellow; femora dark brown, the tips blackened; tibiæ and tarsi black. (A single leg, fore, remains, this with a single tibial spur.) Wings (Plate 1, fig. 1) broad; ground color cream-yellow, cells C and Sc somewhat deeper yellow; the ground largely obscured by broad brown seams to the veins and by longitudinal streaks in the centers of cells R, M, distal half of Cu, and the anal cells, that in 1st A widened outwardly; stigma dark brown, conspicuous; whitish obliterative areas before stigma and across cell 1st M_2; veins brown. Macrotrichia of cells very numerous, including the entire wing excepting the restricted bases of cells R, M, Cu, and 1st A (shown by stippled dots in the figure); cell Cu_1 without macrotrichia except beyond level of m-cu; abundant macrotrichia in the prearcular subcostal cell. Macrotrichia of veins long and conspicuous, including all longitudinal veins, deflections, and crossveins, as m, r-m, and m-cu; a series of trichia on basal portion of the semiatrophied vein Cu_2. Squama with a few long setæ that are scarcely to be differentiated from those of the anal fringe. Venation: Rs long; R_{1+2} entire; m-cu elongate; petiole of cell M_1 shorter than or about equal to m; cell 2d A of moderate width.

Abdominal tergites black, the caudal margins and midline of the intermediate segments yellowish; sternites more uniformly reddish yellow; outer segments, including hypopygium, dark brown. Male hypopygium (Plate 2, fig. 23) relatively small and generalized in structure. Basistyle, *b*, separated from the sternite, 9*s*, by a wide ventral suture, at near apex constricted and then widened into a glabrous portion that bears the dististyles. Ninth tergite (Plate 2, fig. 24, 9*t*) separated from the sternite by a membranous line on about the outer three-fourths, but entirely fused on cephalic portion; caudal portion of tergite not or only slightly projecting beyond the general level of the basistyles; caudal margin, as viewed from above, subtruncate, weakly lobed; median region with three decurved blackened teeth, the median one much smaller than the laterals.

Ninth sternite (Plate 2, fig. 25, 9*s*) very widely membranous beneath, with a narrow median notch, the ventral caudal angles produced into slender hairy lobes. Outer dististyle (Plate 2, fig. 26, *od*) relatively narrow, gently arcuated. Inner dististyle (Plate 2, fig. 26, *id*) with the posterior portion or "heel" terminating in a flattened oval glabrous blade, the cephalic portion a very slender blackened spine.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 11,000 feet, July 20, 1931 (*Franck*).

The very remarkable *Tipula* here described requires comparison with none of the known regional species of the complex. The presence of macrotrichia on all the veins and in virtually all the cells of the wing, including a prearcular group in cell Sc, is elsewhere unknown in the genus. I hesitate to propose a new subgeneric group on this insufficient material and prefer to place it, tentatively at least, in *Trichotipula* Alexander, the other known species of which are Nearctic. The degree of trichiation of the cells of the wing exceeds that of the better-known limoniine genera having hairy wings, as *Ula, Adelphomyia, Ulomorpha,* and even *Ormosia.*

TIPULA MUTILOIDES sp. nov. Plate 1, fig. 2; Plate 2, figs. 27, 28, 29, 30.

Allied to *mutila;* general coloration of mesonotum light gray, the præscutum with three darker gray stripes; setigerous punctures conspicuous; basal segments of antennæ light yellow, the remainder black; legs black, the femoral bases narrowly yellow; wings pale brown, variegated with large whitish areas; R_{1+2} lacking; abdomen yellow, the basal tergites trivittate with black, the outer segments more uniformly blackened; male hypopygium with the tergite divided by a narrow membranous pale line; eighth sternite projecting as a shovel-shaped structure.

Male.—Length, about 12 millimeters; wing, 11.5.

Frontal prolongation of head gray; nasus short and stout; palpi black. Antennæ of moderate length, if bent backward extending about to the base of abdomen; scape and pedicel light yellow, flagellum black, the base of first segment paler; basal enlargement of flagellar segments only moderately developed; verticils shorter than the segments; terminal segment small, subconical. Head gray.

Mesonotal præscutum light gray, with three darker gray stripes, the median stripe with a scarcely evident darker median vitta; interspaces with conspicuous brown setigerous punctures;

posterior sclerites of mesonotum light gray. Pleura, including the dorsopleural membrane, gray. Halteres buffy, the knobs dark brown. Legs with the coxæ gray; trochanters yellow; remainder of legs black, the femoral bases narrowly yellow; tibial spur formula 1–2–2; spurs long and straight, hairy; claws with basal tooth, the proximal half of each claw hairy. Wings (Plate 1, fig. 2) with the ground color pale brown, the prearcular region and cells C and Sc more yellowish; stigmal region somewhat darker brown; conspicuous whitish areas on wing disk, as follows: At near midlength of Rs, in both cells R_1 and R; an incomplete crossband beyond the cord, including cells R_2 to 1st M_2; a large suboval white area at near three-fourths the length of cell M; bases of cells Cu and 1st A whitened; veins brown, paler in the flavous areas. Macrotrichia of veins relatively long and conspicuous, including practically complete series on veins beyond cord; vein 1st A with only two or three trichia at extreme outer end; vein 2d A with trichia on distal two-thirds; Rs without trichia; squama bare. Venation: R_{1+2} atrophied; R3 elongate, nearly twice R_{2+3}; cell 1st M_2 relatively small, pentagonal.

Abdominal tergites light yellow, narrowly trivittate with black; on fifth and succeeding segments more extensively darkened so as to obscure the yellow color; basal sternites more uniformly yellow, the outer segments blackened. Male hypopygium (Plate 2, fig. 27) with the tergite, 9*t*, entirely separate from the sternite, 9*s*. Basistyle, *b*, almost completely separated from sternite by a suture that is broken near its ventral portion for about one-fourth its length. Ninth tergite (Plate 2, fig. 28, 9*t*) extensive, heavily blackened, entirely divided by a pale median line; apex of tergite more narrowed, the caudal margin with a very shallow V-shaped notch; dorsal surface of tergite with numerous small setæ. Outer dististyle (Plate 2, fig. 29, *od*) a very slender elongate rod. Inner dististyle (Plate 2, fig. 29, *id*) relatively narrow, the base projecting caudad as a densely setiferous lobule. Ninth sternite with a dense brush of black setæ lying in the sheathing concavity of the eighth sternite, the latter (Plate 2, fig. 30, 8*s*) projecting caudad as a prow-shaped or shovel-like structure, narrowed caudally, the apex narrowly pale and provided with numerous setigerous punctures.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 11,000 feet, July 29, 1931 (*Franck*).

Tipula mutiloides is allied to species such as *T. futilis* Alexander (Japan), *T. edwardsella* Alexander (Formosa), and others in the eastern Palæarctic fauna, differing most evidently in the structure of the male hypopygium and details of coloration of the body.

MACGREGOROMYIA SZECHWANENSIS sp. nov. Plate 1, fig. 3; Plate 2, figs. 31, 32.

Mesonotal præscutum buffy gray, with three brown stripes; antennæ relatively long; legs with tibiæ and tarsi black; wings with a brownish tinge, stigma and a broad seam on anterior cord dark brown; forks of medial field relatively shallow, cell M_1 less than twice the length of cell 1st M_2; inner dististyle of male hypopygium with the apical beak long and slender.

Male.—Length, about 8 millimeters; wing, 8.5.

Frontal prolongation of head short, buffy brown; palpi dark. Antennæ (Plate 2, fig. 31) with the scape, pedicel, and basal half of first flagellar segment light yellow, the remainder of organ black; flagellar segments long, cylindrical, with short verticils; basal enlargements of segments only slightly developed; flagellar segments beyond the second gradually decreasing in length. Head dark brown.

Mesonotal præscutum buffy gray with three brown stripes; posterior sclerites of mesonotum dark brown, the postnotum sparsely pruinose. Pleura buffy, variegated with dark brown on anepisternum, ventral sternopleurite, and meron. Halteres pale, the knobs infuscated. Legs with the coxæ pale, slightly infuscated basally; trochanters yellow; femora dark brown, the tips blackened; tibiæ and tarsi black. Wings (Plate 1, fig. 3) with a brown tinge, the prearcular and costal regions more yellowish; stigma and a broad seam on anterior cord dark brown; posterior cord and adjoining veins narrowly seamed with brown; more whitish areas before and beyond the stigma; veins dark brown. Macrotrichia of veins relatively long and conspicuous; a few macrotrichia in outer ends of cells R_3 and M_1. Venation: Sc_1 present; Rs with basal section transverse, in alignment with r-m, the second section of Rs short; R_{1+2} lacking; forks of medial cells relatively shallow; cell M_1 less than twice the length of cell 1st M_2.

Abdominal segments buffy brown, the basal ring narrowly blackened; outer segments, including hypopygium, more uniformly infuscated. Male hypopygium (Plate 2, fig. 32) with the lobes of the ninth tergite, 9*t*, separated by a narrow notch,

each lobe not evenly rounded at apex but provided with a very shallow notch on outer margin. Outer dististyle, *od*, long-oval, narrowed to the blunt apex. Inner dististyle, *id*, with the apical beak long and slender.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

The discovery of two new species of *Macgregoromyia* in western China is a matter of unusual interest, the only other species so far discovered being two that occur in the high mountains of northern Luzon. The two species described at this time are more closely related to *M. brevisector* Alexander, than they are to the genotype, *M. benguetensis* Alexander, having the short, perpendicular, basal section of the radial sector of the former species. The Chinese species differ most evidently in the uniformly blackened tibiæ and other details of coloration of the body and wings; *M. szechwanensis* is more generalized in structure than is either *M. brevisector* or *M. celestia* sp. nov.

MACGREGOROMYIA CELESTIA sp. nov. Plate 1, fig. 4; Plate 2, figs. 33, 34.

General coloration of mesonotum brown, the præscutum with three darker brown stripes; antennæ (male) short, the basal segments yellow, the remainder black; flagellar segments without basal enlargements and with very much reduced verticils that are placed at near midlength of the segments; legs brown; wings with a faint brownish tinge, the stigma and seams along cord, vein Cu_1, and marginally from stigma to wing apex brown; cell 1st M_2 small, about one-fourth as long as cell M_1; male hypopygium with the inner dististyle short and broad, with a small apical beak.

Male.—Length, about 11 millimeters; wing, 11.

Frontal prolongation of head light brown; nasus short but evident; palpi with the basal two segments brownish black, the third segment dark brown, the terminal segment paling to yellow. Antennæ (Plate 2, fig. 33) short; scape, pedicel, and first flagellar segment yellow, the remainder black; flagellar segments cylindrical, without indication of basal swelling, the segments gradually decreasing in length outwardly; verticils very small to scarcely evident, placed at near midlength of the segment; antennæ considerably shorter than in *szechwanensis*. Head light brown.

Mesonotum brown, the præscutum with three darker brown stripes, the median one becoming paler behind; humeral and

lateral portions of præscutum somewhat more grayish; scutal lobes and scutellum dark brown. Pleura brown, darker on the ventral anepisternum. Halteres long, the stem pale, the knobs dark brown. Legs very long and slender; coxæ darkened basally, their apices light yellow, most extensive on the posterior coxæ; trochanters pale yellow; remainder of legs brown, the femoral bases slightly brightened; claws small and nearly simple, each bearing a broad tooth near base. Wings (Plate 1, fig. 4) with a faint brownish tinge, cells C and Sc more yellowish brown; stigma oval, dark brown; a clearly defined brown seam along cord; narrow seams along vein Cu and in outer end of cell R_2, extending along wing margin to beyond apex; veins black. Macrotrichia of veins beyond cord long and conspicuous; anal veins without trichia; no trichia in cells of wings. Venation: Sc_1 apparently lacking; Rs with basal section nearly perpendicular, fully twice the second section; R_{1+2} preserved as a short, erect spur; cell 1st M_2 very small, only about one-fourth as long as cell M_1.

Abdominal tergites chiefly dark brown, a trifle more brightened sublaterally just before caudal margin; sternites yellow, with a narrow brown basal ring; outer abdominal segments, including hypopygium, more uniformly dark brown. Male hypopygium having the general structure of the genus; inner dististyle (Plate 2, fig. 34, *id*) of quite different conformation from that of *szechwanensis*, being broad, its apical beak small.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

Macgregoromyia celestia is readily told from *M. szechwanensis* sp. nov. by the larger size, short antennæ, and very different wing venation, cell 1st M_2 being unusually small when compared to cell M_1. In its general appearance, the present fly is somewhat more like *M. brevisector* Alexander (Luzon), yet quite distinct in the venation and in the pattern of the legs and wings.

LIMONIINÆ

LIMONIINI

LIMONIA (LIMONIA) FRANCKI sp. nov. Plate 1, fig. 5; Plate 2, fig. 35.

General coloration gray, the præscutum with a median brown stripe; knobs of halteres orange; legs black, only the femoral bases narrowly light yellow; wings with a heavy brown pattern, cells C and Sc uniformly infuscated; m-cu more than its own length before the fork of M; male hypopygium with the tergite

profoundly split by a median incision, the lobes densely hairy; ventromesal lobe of basistyle very long and conspicuous; mesal-apical lobe of gonapophysis long and slender.

Male.—Length, about 7 millimeters; wing, 8.2.

Female.—Length, about 7 millimeters; wing, 7.8.

Rostrum and palpi black. Antennæ black throughout; flagellar segments oval, with short, inconspicuous verticils; terminal segment from one-third to one-fourth longer than the penultimate. Head brownish gray, the center of vertex somewhat darker; anterior vertex a little less than twice the diameter of the scape.

Pronotum dark brown. Mesonotum gray, the præscutum with a conspicuous dark brown median stripe that becomes obsolete before the suture; lateral stripes obsolete or nearly so. Pleura gray, sparsely variegated with darker areas where the bloom has been denuded. Halteres pale, the knobs orange. Legs with the coxæ gray, vaguely brightened at tips; trochanters yellow; femora black, the bases narrowly light yellow, this equaling about one-fifth to one-sixth the total length of the segment; tibiæ and tarsi black; claws long and slender, with a long basal spine and microscopic denticles at extreme base. Wings (Plate 1, fig. 5) relatively narrow; ground color pale yellow, the prearcular region more intensely yellow; cells C and Sc uniformly dark brown; a heavy solidly dark brown pattern, distributed as follows: A quadrate area at origin of Rs, entirely crossing cell M; a very extensive seam along cord, extending from the stigma entirely across the wing; outer end of cell 1st M_2 narrowly seamed; extensive darkenings in outer ends of anal cells, most extensive in 2d A where the outer two-thirds of the cell is included; wing margin narrowly seamed with brown; veins brown. Venation: Sc_1 ending about opposite two-fifths the length of Rs, Sc_2 close to its tip; free tip of Sc_2 and R_2 in transverse alignment; R_{4+5} elongate, subequal to or exceeding R_{2+3}; cell 1st M_2 about as long as vein M_{1+2} beyond it; m-cu a little more than its own length before the fork of M; cell 2d A of moderate width, the anal veins parallel at origin.

Abdomen black, gray pruinose, the basal sternite more yellowish; caudal margins of sternites narrowly ringed with pale. Male hypopygium (Plate 2, fig. 35) with the tergite, 9*t*, profoundly divided by a linear median incision, the very broad lobes thus formed darkened and provided with very numerous, long, coarse setæ that are more abundant near the midline, becoming fewer toward the lateral margin. Basistyle, *b*, rela-

tively small but with a very large ventromesal lobe that in a position of rest on a microscope slide extends caudad almost to the apex of the ventral dististyle, provided with numerous setæ, those of mesal face near apex very long, erect, light yellow. Ventral dististyle, *vd*, fleshy, the rostral prolongation pendant, narrowed apically, the two rostral spines placed at its base, nearly straight, lying flat against surface of style. Gonapophyses, *g*, with the mesal-apical lobes narrowed to a slender, gently curved spine.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 11,000 feet, July 18, 1931 (*Franck*). Allotopotype, female, altitude 11,000 feet, July 30, 1931.

This very distinct fly is named in honor of the collector as a slight appreciation of his efforts in making known the crane flies of western China. The unusual male hypopygium, in conjunction with the basal position of m-cu, furnishes characters that render it unnecessary to compare the fly with any of its distant relatives. The structure of the rostral prolongation of the male hypopygium vaguely suggests that of *Limonia* (*Dicranomyia*) *sordida* (Brunetti) and allies, but the present species is a member of the typical subgenus *Limonia*.

LIMONIA (LIMONIA) LATICELLULA sp. nov. Plate 1, fig. 6.

General coloration gray, the præscutum with three dark brown stripes; antennæ black throughout, the basal flagellar segments subglobular; femora yellowish brown, the tips narrowly blackened; wings broad, whitish, this color including the broad prearcular region, the disk heavily patterned with brown and gray; m-cu oblique in position, placed a short distance before fork of M; cell 2d A very broad.

Female.—Length, about 7 to 9 millimeters; wing, 7.2 to 11.

Rostrum black, pruinose; palpi black. Antennæ black throughout; basal flagellar segments subglobular to very short-oval, the outer segments passing to oval; verticils shorter than the segments. Head blackish, sparsely pruinose; anterior vertex narrow.

Mesonotum gray, the præscutum with three dark brown stripes, the median one not reaching the suture; scutal lobes dark brown. Pleura gray, this color including the dorsopleural region. Halteres whitish. Legs with the coxæ dark, gray pruinose; femora obscure yellow, the tips narrowly but conspicuously blackened, the amount not exceeding the distal eighth of the segment; tibiæ brownish yellow, the tips narrowly brownish black,

the degree of darkening less than that of the femoral tips; tarsi yellow to brownish yellow, the outer segments dark brown. In the Pehlinting paratype, the femoral tips are a little broader and the tibiæ more uniformly darkened. Wings (Plate 1, fig. 6) broad, the ground color white, including the broad prearcular region; a heavy brown and gray pattern, distributed as follows: Bases of cells C and Sc darkened, the latter more extensively so; brown areas at arculus, origin of Rs, at fork of Sc, stigma, along cord, and on outer end of cell 1st M_2; vague, paler, more-grayish clouds in the cubital and outer radial and medial cells; very large, grayish brown areas in the outer ends of both anal cells, that in cell 2d A being so extensive as to include all but the basal third of cell; veins dark brown, costa more yellowish, the prearcular veins paler. Venation: Sc_1 ending about opposite midlength of Rs, Sc_2 near its tip; Rs angulated and sometimes spurred at origin (including type); free tip of Sc_2 and R_2 in transverse alignment; m-cu oblique, approximately one-fourth to one-third its own length before fork of M; cell 2d A very wide.

Abdomen brownish black, the margins of the segments narrowly but conspicuously paler, gray. Ovipositor with the cerci slender and nearly straight; all valves dark reddish horn-color.

Habitat.—China (Szechwan).

Holotype, female, Mount Omei, altitude 11,000 feet, July 18, 1931 (*Franck*). Paratopotypes, 2 females, altitude 7,000 feet, July 17, 1931; 1 female, Pehlinting, Mount Omei, altitude 6,000 feet, July 1931.

The nearest ally of the present fly seems to be *Limonia* (*Limonia*) *francki* sp. nov., which has a somewhat similar wing pattern but is readily told by the blackened legs, the narrow wings, with m-cu far before the fork of M, and other characters. The Pehlinting paratype of the present species is much smaller than the type and differs somewhat in the coloration of the legs, as described.

LIMONIA (LIMONIA) BICORNIGERA sp. nov. Plate 1, fig. 7; Plate 2, fig. 36.

General coloration reddish yellow, the thoracic pleura with a dark brown longitudinal stripe; antennal segments with short, glabrous, apical necks; femora brownish black, the tibiæ and tarsi paler brown; wings tinged with brown; Sc_1 ending just beyond midlength of Rs; male hypopygium with the dististyle single, its rostral prolongation developed into a powerful, blackened, spinelike rod that narrows to an acute tip.

Male.—Length, about 5 millimeters; wing, 5.8.

Female.—Length, about 5.5 millimeters; wing, 6.2.

Rostrum dark brown; palpi brownish black. Antennæ black throughout; flagellar segments short-oval, becoming smaller and more elongate-oval outwardly, the segments with short, stout, glabrous, apical necks; verticils shorter than the segments; terminal segment a little longer than the penultimate. Head gray, the anterior vertex reduced to a linear strip in both sexes.

Pronotum dark brown. Mesonotum light reddish yellow to yellowish testaceous, without distinct markings. Pleura yellow, with a conspicuous, dark brown, longitudinal stripe extending from the propleura across the central sclerites, passing beneath the wing root to abdomen. Halteres pale yellow, the knobs infuscated. Legs with the coxæ and trochanters yellow; femora brownish black, the bases obscure yellow; in female, the femora more extensively pale; tibiæ and tarsi light brown, the outer tarsal segments darker; claws with a single, slender, subbasal spine, with additional more basal spinous setæ. Wings (Plate 1, fig. 7) with a brownish tinge; stigma subcircular, darker brown; veins brown. Venation: Sc_1 ending just beyond mid-length of Rs, Sc_2 at its tip; free tip of Sc_2 and R_2 in approximate transverse alignment; m-cu close to fork of M; anal veins gently convergent beyond level of anal crossvein, thence sinuous to margin.

Abdomen brownish black, the basal sternites more brownish yellow; hypopygium dark. Male hypopygium (Plate 2, fig. 36) with the caudal margin of tergite, 9*t*, gently emarginate. Basistyle, *b*, with the ventromesal lobe broad. A single dististyle, *d*, the body of which is a small oval setiferous lobe; rostral prolongation a powerful, heavily sclerotized spinelike rod that gradually narrows to a gently decurved acute point; surface of prolongation with setæ scattered over its entire length but without rostral spines. Gonapophyses, *g*, blackened and unequally bifid at tips. Apical lateral angles of ædeagus prolonged into narrow points. Ovipositor with the cerci relatively small, slender, strongly upcurved; hypovalvæ powerful, straight, dark horn-color, blackened basally.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*). Allotopotype, female. Paratopotype, female.

Limonia (*Limonia*) *bicornigera* is most nearly allied to the eastern Asiatic *L.* (*L.*) *machidai* (Alexander), agreeing in the

general appearance and fundamentals of structure of the hypopygium. It differs from this species and from all other members of the subgenus known to me by the powerful, acutely pointed, rostral prolongation of the dististyle.

LIMONIA (DICRANOMYIA) SUBLIMIS sp. nov. Plate 1, fig. 8; Plate 2, fig. 37.

Belongs to the *pulchripennis* group; general coloration black, the thorax variegated by areas of yellowish gray pollen; wings white, with a heavy brown pattern, including about five costal areas that are solidly darkened in the costal and subcostal fields but with conspicuous white centers in cell R; male hypopygium with two spines on the rostral prolongation of the ventral dististyle.

Male.—Length, about 7 millimeters; wing, 8.3.

Female.—Length, about 8 millimeters; wing, 8.5.

Rostrum and palpi black. Antennæ with the scape black, the remaining segments dark brown; flagellar segments oval, the outer segments more elongate-oval, the verticils subequal to or shorter than the segments. Head grayish brown, the narrow anterior vertex more yellowish gray; a blackish area on either side of the narrow median line on the anterior portion of the posterior vertex.

Mesonotal præscutum yellowish gray, with three conspicuous black stripes, the median stripe broad and complete, the lateral stripes small; lateral margins of sclerite blackened; posterior sclerites of mesonotum blackish. Pleura variegated with black and yellowish gray pollinose areas. Halteres almost white, the knobs blackened. Legs with the coxæ and trochanters black; femora yellow, the tips broadly and conspicuously blackened; tibiæ yellow, the bases blackened, the amount about equal to one-half the femoral apex; tips of tibiæ blackened; tarsi black, the proximal third of basitarsi paler; claws long, with a single slender basal spine, with additional microscopic denticles nearer the base. Wings (Plate 1, fig. 8) with the ground color white, the prearcular region light yellow; a very heavy brown pattern, including about five costal areas that are much wider than the interspaces, the third area at origin of Rs, the fourth at stigma; all costal areas solidly darkened in cells C and Sc, the first four with conspicuous white centers where they occupy the radial field; costal darkenings not narrowed posteriorly (as is the case in *frivola* and *shirakii*); wing apex solidly but not quite so intensely darkened; narrower clouds along cord and outer end of cell 1st M_2; more grayish brown clouds in medial field and as large areas at ends of veins Cu_1, 1st A, and 2d A, the latter

more intensely darkened at their margins; a further pale gray wash across the middle of the anal cells; veins pale, brown in the darkened areas. Venation: Sc_1 ending about opposite one-third to one-fourth the length of Rs, Sc_2 apparently lacking; m-cu about one-third or more of its length before the fork of M; cell 2d A of moderate width.

Abdomen black, the caudal margins of the segments very narrowly paler; hypopygium dark. Male hypopygium (Plate 2, fig. 37) with the rostral prolongation of the ventral dististyle, *vd*, very short and stout, with two spines that exceed in length the entire prolongation, these spines placed close together on the summit of the prolongation. Gonapophyses, *g*, with the mesal-apical lobe relatively slender and gently curved.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 11,000 feet, July 18, 1931 (*Franck*). Allotopotype, female, altitude 11,000 feet, July 17, 1931.

The *pulchripennis* group of *Dicranomyia* includes several unusually handsome species, all having the body coloration black, variegated with yellowish gray, the knobs of halteres blackened, the tips of femora and bases and tips of tibiæ conspicuously blackened, and with a heavy brown wing pattern that includes several costal areas. The group divides into two subgroups, of which the typical one, including *pulchripennis* (Brunetti), of northern India and western China, and *subpulchripennis* Alexander, of western China, have the intermediate dark marginal areas of the front part of wing variegated by pale centers in the costal cell and have a single elongate spine on the rostral prolongation of the male hypopygium. The second subgroup, including the present fly, *frivola* Alexander, of Formosa, *kirishimana* Alexander, of southern Japan, and *shirakii* (Alexander), of Formosa, have the marginal areas solidly darkened in cell C and with two spines on the rostral prolongation of the male hypopygium. The present fly is closest to *kirishimana* in its general characters, differing most evidently in the larger size, clear white centers of the intermediate costal areas of the wing, where these latter occupy cell R, and by the narrowly darkened seams to the clouded areas along the anal margin of wing.

HELIUS (HELIUS) INFIRMUS sp. nov. Plate 1, fig. 9; Plate 3, fig. 38.

Allied to *tenuistylus;* general coloration dark brown; legs dark brown, the tarsi paling to brownish yellow; wings with a strong grayish brown suffusion, the stigma darker; costal

fringe (male) short; Rs only a little shorter than its anterior branch.

Male.—Length, about 7.5 millimeters; wing, 7.

Rostrum about one-fourth longer than the remainder of head, brownish black; palpi black. Antennæ short, black throughout; flagellar segments oval, becoming more elongate-oval outwardly; outer verticils much exceeding the segments. Head blackish.

Pronotum, mesonotum, and pleura dark brown, the ventral pleurites a little more brightened. Halteres dark brown. Legs with the coxæ and trochanters brownish testaceous; femora and tibiæ chiefly dark brown; narrow tips of tibiæ and most of tarsi paling to brownish yellow. Wings (Plate 1, fig. 9) with a strong grayish brown tinge, cells C and Sc somewhat darker; stigma elongate, still darker brown; veins brownish black. Costal fringe (male) short. Venation: Sc_1 ending just before the fork of Rs, Sc_2 near its tip; Rs relatively long, only a little shorter than its anterior branch; second section of M_{1+2} a little longer than the oblique m, so the inner end of cell 2d M_2 lies proximad of that of cell M_3; m-cu shortly before fork of M.

Abdominal tergites brownish black, the basal sternites obscure brownish yellow, the outer segments darker. Male hypopygium (Plate 3, fig. 38) with the mesal face of basistyle, *b*, near base with a conspicuous spiniferous lobe. Outer dististyle, *od*, gently curved, blackened, the apex very indistinctly toothed.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, July 17, 1931 (*Franck*).

Helius (*Helius*) *infirmus* is most nearly allied to *H.* (*H.*) *costofimbriatus* Alexander (Riukiu Islands) and *H.* (*H.*) *tenuistylus* Alexander (Formosa) in the venation and general structure of the male hypopygium. It differs from the former in the short costal fringe of the male sex; from the latter species it differs chiefly in the long Rs and slight details of the male hypopygium.

ANTOCHA (ANTOCHA) CONSTRICTA sp. nov. Plate 1, fig. 10; Plate 3, fig. 39.

General coloration gray; antennæ dark brown throughout; flagellar segments elongate-oval, the outer ones becoming smaller; femora yellow, the tips infuscated; wings milky white, the stigma barely indicated; cell 1st M_2 long, m-cu at fork of M; male hypopygium with each gonapophysis bearing a small lateral spine near apex; arms of phallosome powerful, each bearing a strong curved spine on mesal face at midlength.

Male.—Length, about 5 to 5.3 millimeters; wing, 6 to 6.2.

Rostrum and palpi brown. Antennæ dark brown throughout; flagellar segments elongate-oval, the outer segments becoming smaller and shorter, the terminal segment about two-thirds as long as the penultimate; verticils small and inconspicuous. Head light gray.

Pronotum brownish yellow, narrowly dark brown medially. Mesonotum gray, the humeral region restrictedly obscure yellow. Pleura, including pleurotergite, obscure yellow, very sparsely pruinose. Halteres pale, the knobs weakly infuscated. Legs with the coxæ and trochanters obscure yellow, the fore coxæ a trifle darker; femora yellow, the tips rather broadly and conspicuously infuscated; tibiæ yellow, the tips insensibly darkened; tarsi yellow, the outer segments brown. Wings (Plate 1, fig. 10) milky white, the prearcular region even clearer white; stigma barely indicated; veins brown, paler at the wing base. Anal angle of wing moderately developed. Venation: R_{2+3} only a trifle longer than R_{4+5}; cell 1st M_2 long, exceeding in length any of the veins beyond it; m-cu at fork of M, the distal section of Cu_1 more than one-third longer than m-cu.

Abdominal tergites brown, the basal sternites more yellowish; hypopygium dark. Male hypopygium (Plate 3, fig. 39) with the tergite, 9*t*, transverse, unusually narrow, the posterior margin constricted at near midlength, the greatest width (transverse) being about six times the length at narrowest point (longitudinal); tergal setæ numerous but confined to the caudal half of the segment, the more lateral setæ larger and powerful. Dististyles nearly terminal in position, the outer, *od*, shorter, the distal half heavily blackened, the apex obliquely truncated so the lower apical angle is subacute. Gonapophyses, *g*, long and slender, pale, before apex on outer margin with a slender spine that is not more than one-half as long as the stouter axial point. Phallosome, *p*, appearing as a somewhat lyrate plate, each arm stout and powerful, at near midlength on mesal face bearing a stout curved spine; outer margin near this same point with a small tubercle, larger and more conspicuous in the paratype than in the holotype figured.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*). Paratopotype, male.

The branched gonapophyses and arms of phallosome of the hypopygium separate the present species from all regional allies,

with the exception of *Antocha* (*Antocha*) *multidentata* sp. nov., which is most readily told by genitalic characters.

ANTOCHA (ANTOCHA) MULTIDENTATA sp. nov. Plate 3, fig. 40.

General coloration of mesonotum brown, sparsely pruinose, the lateral margins of the præscutum obscure yellow; antennæ dark throughout; knobs of halteres weakly infumed; legs obscure yellow, the outer tarsal segments darkened; wings whitish subhyaline, sparsely variegated with darker; m-cu close to fork of M; male hypopygium with both the gonapophyses and lateral arms of phallosome bearing small lateral spines.

Male.—Length, about 4.5 to 5.2 millimeters; wing, 5 to 5.8.

Female.—Length, about 6 millimeters; wing, 6.5.

Rostrum light brown; palpi brown. Antennæ dark brown throughout; flagellar segments oval, with very small, inconspicuous verticils. Head dark brownish gray.

Mesonotal præscutum chiefly covered by three brown stripes that are confluent or nearly so, the lateral margins and median area before the suture obscure yellow; posterior sclerites of notum dark brown, sparsely pruinose. Pleura obscure yellow, vaguely marked with darker on the ventral sternopleurite. Halteres pale, the knobs weakly infuscated. Legs with the fore coxæ darkened, the remaining coxæ and all trochanters yellow; remainder of legs obscure yellow, the outer tarsal segments more infuscated. Wings whitish subhyaline, the stigma and vague seams to most of the longitudinal veins slightly darker than the ground color, the seams most evident as darkened veins inclosed by somewhat paler brown margins; costal region clearer yellow; veins pale, those beyond cord, Cu, 2d A, and outer half of 1st A somewhat darker. Venation: R_2 lying opposite or slightly distad of r-m; cell 1st M_2 as long as or slightly longer than the cells beyond it; m-cu at or a very short distance before the fork of M.

Abdominal tergites dark brown, the caudal margins a trifle paler; sternites a little paler brown than the tergites; subterminal segments a trifle darker than the base; hypopygium brownish yellow. Male hypopygium (Plate 3, fig. 40) with the tergite, 9*t*, transverse, the caudal margin gently emarginate. Outer dististyle, *od*, relatively short, the outer end blackened, the apex very obliquely truncated, so the extreme tip is subacute. Gonapophyses, *g*, branched near outer end, the axial spine being longer and stouter than the weak lateral spine, the reverse

of the condition obtaining in species such as *constricta* and *spiralis*. Arms of phallosome, *p*, somewhat lyriform, narrowed to acute points, on margin at about one-third the length bearing a slender acute branch.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 3,500 feet, August 17, 1931 (*Franck*). Allotype, female, altitude 7,000 feet, July 17, 1931. Paratypes, 2 males, with the allotype; 1 male, altitude 3,500 feet, August 16, 1931.

Antocha (*Antocha*) *multidentata* is readily told from all regional species by the structure of the male hypopygium. The most similar species is *A.* (*A.*) *constricta* sp. nov., which has a very different conformation of the outer structures of the phallosome.

ANTOCHA (ANTOCHA) SPIRALIS sp. nov. Plate 3, fig. 41.

General coloration gray; antennæ black throughout, short; halteres and legs brown; wings gray, the prearcular region milky white; abdomen brownish black, the hypopygium brighter; male hypopygium with the gonapophyses branched near tips; lateral arms of phallosome slender, simple, before apex twisted into a complete spiral turn.

Male.—Length, about 4 millimeters; wing, 4.5.

Rostrum yellowish brown; palpi black. Antennæ black throughout, short, if bent backward not attaining the wing root; flagellar segments short-oval, the verticils short and inconspicuous. Head broad, dark gray.

Mesonotum and pleura uniformly dark gray. Halteres brownish black, the base of stem narrowly pale yellow. Legs with the coxæ yellow, the fore and middle coxæ more infuscated; trochanters obscure yellow; femora brown; tibiæ and tarsi dark brown to brownish black. Wings grayish, the prearcular region milky white; veins brown. Venation: Cell 1st M_2 relatively small; m-cu a short distance before the fork of M.

Abdomen brownish black, the disk of the basal two tergites vaguely brighter; hypopygium brownish yellow. Male hypopygium (Plate 3, fig. 41) with the outer dististyle, *od*, moderately sclerotized, the apex obtuse. Gonapophyses, *g*, very slender, with a subterminal spine on outer margin, this much smaller and weaker than the inner or axial branch. Lateral arms of phallosome, *p*, simple, very slender, before apex twisted into a complete spiral turn.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 3,500 feet, August 17, 1931 (*Franck*).

The curious conformation of the lateral arms of the phallosome of the male hypopygium suffices to distinguish the present fly from all of its now numerous regional allies. There are now more than a score of species of *Antocha* in Japan and China, with no fewer than nine distinct forms occurring on the single peak, Mount Omei.

ANTOCHA (ANTOCHA) NIGRIBASIS sp. nov. Plate 1, fig. 11; Plate 3, fig. 42.

General coloration pale yellow, including the head, thorax, abdomen, and halteres; legs yellow, the tips of the femora narrowly brownish black; wings milky white, the prearcular region and areas on the wing disk brownish black; R_2 lying far basad of r-m; m-cu about one and one-half times its own length before the fork of M; male hypopygium with the phallosome asymmetrical, consisting of a single elongate spine subtending the ædeagus.

Male.—Length, about 4.5 to 4.7 millimeters; wing, 5 to 5.3.

Rostrum yellow; palpi pale basally, the outer segments passing into brown. Antennæ short, pale yellow; flagellar segments oval, the longest verticils about equal to the segments. Head pale yellow.

Thorax pale yellow, the scutal lobes very weakly darkened. Halteres pale yellow. Legs pale yellow, the tips of the femora narrowly and conspicuously dark brown to brownish black; in one paratype, the tips of the tibiæ are similarly darkened but somewhat more narrowly so; outer tarsal segments darkened; claws with a small tooth at base. Wings (Plate 1, fig. 11) milky white, handsomely variegated with brownish black, as follows: Prearcular cells; a cloud at origin of Rs; stigma; narrow seams along cord, m-cu, outer end of cell 1st M_2, and along the veins issuing from cell 1st M_2, the dark color beyond cord being indicated mostly by the darkened veins; veins pale, brown in the darkened areas; costal region brighter yellow. Venation: R_2 only a little shorter than R_{2+3}, the latter about one-third R_{4+5}, R_2 thus lying far proximad of r-m; veins issuing from cell 1st M_2 divergent; m-cu more than one and one-half times its own length before the fork of M.

Abdomen yellow. Male hypopygium (Plate 3, fig. 42) with the tergite, 9*t*, transverse, the caudal end gently emarginate and weakly crenulate, with numerous setæ. Dististyles subterminal in position, the outer, *od*, a sclerotized, ribbonlike blade. Gona-

pophyses, *g*, appearing as straight, simple blades, their tips acute. Phallosome, *p*, appearing asymmetrical, consisting of a single curved spine subtending the elongate ædeagus.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,000 feet, July 14, 1931 (*Franck*). Paratopotypes, 2 males, with type; 1 male, August 10, 1931.

Antocha (*Antocha*) *nigribasis* is very distinct from all other described regional species of the genus in the pale yellow coloration of the body, in conjunction with the narrowly blackened tips of the femora, the darkened wing bases, the proximal position of m-cu, and the structure of the male hypopygium.

ANTOCHA (ANTOCHA) BIDENS sp. nov. Plate 1, fig. 12; Plate 3, fig. 43.

General coloration yellow, the præscutum with four brownish gray stripes, the lateral pair crossing the suture onto the lateral portions of the scutal lobes; postnotal mediotergite dark, the cephalic lateral portions pale; pleura and pleurotergite yellow, variegated with brown; wings white, the longitudinal veins conspicuously darkened; distal segments of abdomen dark brown, much darker than the basal segments; male hypopygium with the caudal margin of tergite bilobed; apex of outer dististyle bidentate; gonapophyses and lateral arms of phallosome simple.

Male.—Length, about 4 to 5.5 millimeters; wing, 4.5 to 6.2.

Female.—Length, about 5 to 6.5 millimeters; wing, 5.5 to 7.

Rostrum pale brown; palpi brownish black. Antennæ brown throughout; flagellar segments oval, the verticils shorter than the segments. Head obscure yellow.

Mesonotum obscure yellow, the præscutum with four brownish gray stripes, the intermediate pair contiguous to nearly confluent, ending some distance before the suture; scutum yellow, the lobes darkened by caudal extensions of the lateral præscutal stripes; scutellum dark, with a small median yellow triangle at base; postnotal mediotergite dark, the cephalic-lateral angles paler; pleurotergite pale yellow, the ventral margin dark brown. Pleura yellow, variegated with brown, this color including the ventral anepisternum and the more extensive ventral sternopleurite. Halteres pale, the knobs light yellow. Legs with the fore coxæ infuscated, the remaining coxæ and all trochanters pale yellow; remainder of legs yellow, the outer tarsal segments brownish black; claws each with a basal spine. Wings (Plate 1, fig. 12) white; stigma brown, elongate-oval, the costal margin adjoining stigma more yellowish; longitudinal veins, with the exception of M and basal half of 1st A, narrowly seamed

with brown, the veins being darkened, pale in the ground areas. Venation: Sc_1 ending a short distance before end of Rs; R_2 and r-m in nearly transverse alignment; cell 1st M_2 long, equal to the longest vein beyond it; m-cu at or only a short distance before fork of M.

Male with the abdominal tergites pale brown, with a capillary darker median line; segments 6 to 9, including hypopygium, more uniformly dark brown; female with abdomen more uniformly pale, the segments variegated with brown, the caudal margins narrowly yellow; terminal segments, including ovipositor, darker brown to brownish black. Male hypopygium (Plate 3, fig. 43) with the tergite, *9t*, transverse, the broad median portion produced, at the lateral ends of this area with a small obtuse tubercle; caudal margin of tergite incised medially. Outer dististyle, *od*, flattened, black, the apex unequally bifid. Gonapophyses, *g*, appearing as slender, simple rods, the tips very narrow, acute. Phallosome, *p*, with the lateral arms appearing as flattened blades, the two taken together appearing sublyriform.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 3,500 feet, August 17, 1931 (*Franck*). Allotopotype, female, August 16, 1931. Paratopotypes, 3 males, 4 females, August 16 and 17, 1931.

Antocha (*Antocha*) *bidens* is very distinct from all regional species of the genus. The handsomely variegated wings, in conjunction with certain details of the male hypopygium, as the bituberculate ninth tergite and notched outer dististyle, furnish characters that are distinctive.

HEXATOMINI

ADELPHOMYIA LATISSIMA sp. nov. Plate 1, fig. 13; Plate 3, fig. 44.

General coloration of mesonotal præscutum bright brown, the posterior sclerites of the mesonotum darker brown; antennæ black throughout; legs brownish yellow; wings (male) broad, widest opposite the termination of vein 2d A; macrotrichia of cells very sparse, restricted to about eight in extreme outer ends of cells R_4 and R_5; abdominal tergites dark brown, hypopygium yellow.

Male.—Length, about 4.5 millimeters; wing, 5.5 by 1.8.

Rostrum and palpi brownish black. Antennæ black throughout; basal flagellar segments long-oval, the outer segments more elongate, the verticils exceeding the segments; terminal segment

a little longer than the penultimate. Head dark brown; anterior vertex broad.

Pronotum dark brown. Mesonotal præscutum bright brown, the posterior sclerites of notum darker brown to brownish black. Pleura yellowish brown on ventral sclerites, darker brown dorsally. Halteres weakly infuscated, the base of stem narrowly yellow. Legs with the fore coxæ brownish yellow, the remaining coxæ more testaceous-yellow; trochanters yellow; remainder of legs pale brownish yellow; tibial spurs distinct. Wings (Plate 1, fig. 13) with a pale brownish tinge, the prearcular and costal regions somewhat more brownish yellow; stigma slightly darker brown than the ground; veins pale brown. Wings (male) wide, broadest opposite termination of vein 2d A; macrotrichia of cells restricted to about five in outer end of cell R_4 and about three in outer end of cell R_5; macrotrichia on veins beyond cord, basad of cord being found on R_1, outer three-fourths of Rs, outer portion of M and basal section of Cu_1, distal half of 1st A, but virtually lacking on 2d A. Venation: Sc_1 ending opposite fork of Rs, Sc_2 some distance from its tip, Sc_1 alone being nearly as long as m-cu; Rs weakly angulated at origin; R_2 a little longer than R_{2+3}; r-m long, gently arcuated; cell M_1 small; m-cu before midlength of cell 1st M_2; vein 2d A long.

Abdominal tergites dark brown, the sternites a little paler; hypopygium obscure yellow. Male hypopygium (Plate 3, fig. 44) with the outer face of basistyle, *b*, provided with very long, coarse, black setæ, the longest exceeding one-half the length of style. Outer dististyle, *od*, with two apical teeth, the outermost a little slenderer.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 3,500 feet, August 17, 1931 (*Franck*). Paratopotype, male.

Adelphomyia latissima is most nearly allied to *A. flavescens* (Brunetti) (syn., *furcata* Brunetti) of the eastern Himalayas, differing most evidently in the broad wings of the male and the details of coloration, especially of the antennæ, thorax, and halteres. Both species have the macrotrichia of the wing cells so reduced in number as to indicate the probability of their total disappearance in some still undiscovered species of the genus. The structure of the male hypopygium in this genus is rather distinctive and should suffice to correctly assign any species with the cell macrotrichia greatly reduced or lost.

PHYLLOLABIS PICTIVENA sp. nov. Plate 1, fig. 14.

General coloration black; knobs of halteres infuscated; wings yellow, with most of the longitudinal veins broadly and conspicuously seamed with brown; R_{2+3+4} only gently arcuated; m-cu at about two-thirds the lower face of cell 1st M_2.

Female.—Length, about 7 millimeters; wing, 7.6.

Rostrum and palpi black. Antennæ with the scape black, the pedicel light brown; flagellum dark brown; flagellar segments elongate-oval, the verticils a little shorter than the segments. Head brownish black.

Pronotum, mesonotum, and pleura black. Halteres obscure yellow, the knobs infuscated. Legs with the coxæ brownish black; trochanters light brown; femora black; tibiæ brown, the tips narrowly infuscated; tarsi passing to dark brown. Wings (Plate 1, fig. 14) with a yellow tinge; stigma long-oval, dark brown; conspicuous brown seams on certain of the longitudinal veins, as follows: A very broad seam along vein Cu and the cord; somewhat narrower seams along Rs and all outer branches of the radial and medial fields; vein 2d A narrowly bordered by brown; wing margin and axillary border narrowly infuscated; veins brown, somewhat darker in the clouded areas. Macrotrichia of veins abundant but not of excessive length, occurring on all longitudinal veins excepting the basal sixth of M. Venation: Sc_1 ending about opposite one-third the length of Rs, Sc_2 a short distance from its tip; R_{2+3+4} only gently arcuated; m-cu at about two-thirds the lower face of cell 1st M_2; anterior arculus lacking or represented only by a weak clouding.

Abdomen black; cerci deep horn-color, long, nearly straight, their margins smooth; hypovalvæ pale yellow.

Habitat.—China (Szechwan).

Holotype, female, Mount Omei, altitude 11,000 feet, July 18, 1931 (*Franck*).

Phyllolabis pictivena is very different from the two other described species in the eastern Palæarctic region, *P. beesoni* Alexander and *P. confluenta* Alexander, both of the western Himalayas, in the intense black coloration of the body and in the handsomely patterned wings.

ERIOPTERINI

RHABDOMASTIX (PALÆOGONOMYIA) OMEINA sp. nov. Plate 1, fig. 15.

General coloration brown; antennæ (male) elongate, about two-thirds the length of wing; legs brown, the tarsi whitish; wings with a strong brown tinge; macrotrichia of veins very much reduced in number; Sc_1 ending at near midlength of Rs.

Male.—Wing, 3.5 millimeters; antenna, about 2.5.

Rostrum and palpi dark. Antennæ relatively long, being approximately two-thirds the length of wing, dark brown; flagellar segments long-cylindrical, with abundant erect setæ scattered over the whole surface. Head brown.

Mesonotum and pleura pale brown. Halteres broken. Legs with the coxæ and trochanters testaceous-yellow; a single leg (hind) remains; femora brown; tibiæ brown, somewhat paler apically; tarsi whitish. Wings (Plate 1, fig. 15) with a strong brownish tinge, the stigma not indicated; veins slightly darker brown. Macrotrichia of veins, excluding costa, very sparse and restricted, being confined to R_1 beyond cord, distal half of outer section of R_2 and outer portions of distal sections of M_{1+2} and M_3. Wings strongly narrowed basally, the anal angle greatly reduced. Venation: Sc_1 ending about opposite mid-length of Rs, Sc_2 at near mid-distance between origin of Rs and tip of Sc_1; Rs about one-half longer than R_{2+3+4}; space on costa between R_{1+2} and R_3 longer than the latter vein.

Abdomen broken.

Habitat.—China (Szechwan).

Holotype, a broken male, Mount Omei, altitude 4,000 feet, July 4, 1931 (*Franck*).

I am referring the present fly to the subgenus *Palæogonomyia* Meunier,[2] which has hitherto been known only as fossil in Baltic amber (Lower Oligocene). However, the distinctions between the three proposed subgenera, *Rhabdomastix* Skuse, *Palæogonomyia,* and *Sacandaga* Alexander, are so slight that the value of the present reference from a distributional standpoint must be held as being doubtful. It seems certain that the present species is more closely allied to the fossil species placed in *Palæogonomyia* than it is to any other described recent member of the genus. *Palæogonomyia* has the antennæ of the male elongate but still shorter than the wing, whereas the organ is very much longer in the typical subgenus while being very short in both sexes in *Sacandaga.*

GNOPHOMYIA COLLATA sp. nov. Plate 1, fig. 16.

General coloration of mesonotum black; head dark gray; halteres pale, the knobs dark brown; femora brownish yellow, the tips more infuscated; wings with a pale brownish tinge; macro-

[2] Meunier, F., Bull. Soc. Ent. France for 1899 (1899) 359; Ann. des sciences natur., Zoöl. IX 4 (1906) 372.—Alexander, C. P., Bernstein-Forschungen Heft 2 (1931) 111–117.

trichia of basal costal fringe very long and conspicuous; m-cu about one-half its length beyond fork of M; genital shield and cerci dark brown.

Female.—Length, about 7.5 millimeters; wing, 7.

Rostrum and palpi brownish black. Antennæ with the scape and pedicel dark brown, the flagellum black; flagellar segments long-oval to subfusiform, the longest verticils a little exceeding the segments. Head dark gray.

Pronotum and mesonotum black, the anterior lateral pretergites narrowly light yellow. Pleura with the dorsal sclerites black, the meral region much paler, obscure yellow. Halteres pale, the knobs dark brown. Legs with the fore coxæ dark brown, the middle and posterior coxæ yellow; trochanters brownish yellow; femora and tibiæ brownish yellow, the tips more infuscated; tarsi brownish black. Wings (Plate 1, fig. 16) uniformly tinged with pale brown; veins and macrotrichia dark brown; a very conspicuous obliterative streak crosses the basal sections of M_{1+2} and M_3 at both ends of cell 1st M_2. Macrotrichia of costal fringe very long on basal third of wing, becoming shorter and denser on outer portion of costa. Venation: Sc_1 ending just before fork of R_{2+3+4}, Sc_2 shortly beyond r-m; R_{2+3} exceeding two times R_2 alone; r-m connecting with Rs some distance before the fork; cell 1st M_2 small, its proximal end lying distad of that of cell R_5; cell 2d M_2 fully three times as long as cell 1st M_2; m-cu about one-half its length beyond the fork of M.

Abdominal tergites dark brown, the sternites pale yellow. Ovipositor with the genital shield and cerci dark brown.

Habitat.—China (Szechwan).

Holotype, female, Mount Omei, altitude 4,000 feet, August 14, 1931 (*Franck*).

The closest ally of the present species is *G. brevicellula* Alexander (Formosa), which is most readily told by slight differences in the coloration of the body, halteres, legs, and wings, and the details of venation. Both species have r-m connecting with Rs some distance before its fork and with cell 2d M_2 fully three times as long as cell 1st M_2. *Gnophomyia brevicellula* has a very remarkable hypopygium, and it will be of great interest to discover the male of the present species for comparison.

DASYMALLOMYIA PERSIGNATA sp. nov. Plate 1, fig. 17; Plate 3, fig. 45.

Mesonotal præscutum yellow, variegated with shiny black and chestnut areas; wings light yellow, with incomplete crossbands at level of origin of Rs and along cord; a cloud at fork

of M_{2+3}; vein R_3 nearly one-half of R_4; male hypopygium with the outer dististyle bearing a median rounded tubercle that is set with coarse setæ.

Male.—Length, about 5 to 5.5 millimeters; wing, 5.3 to 5.5.

Female.—Length, about 6 millimeters; wing, 6.1.

Rostrum brown; palpi black. Antennæ bicolorous, the segments black, the incisures narrowly obscure yellow; flagellar segments oval, becoming more elongate outwardly, the verticils long and conspicuous, exceeding the segments. Head brown.

Mesonotal præscutum polished light yellow, handsomely patterned with black and chestnut, the sublateral areas being two black crossbars alternating with chestnut; median black stripe bordered centrally with chestnut; scutum yellow medially, the lobes polished black; scutellum infuscated, obscure yellow behind; postnotum brownish black. Pluera light yellow, variegated with large black areas. Halteres light yellow, the knobs broken. Legs with the coxæ brown; trochanters obscure yellow; femora yellow, with a narrow black subterminal ring; tibiæ yellow, narrowly tipped with black; tarsi yellow, the outer segments brownish black; legs conspicuously hairy. Wings (Plate 1, fig. 17) light yellow, the prearcular and costal regions somewhat deeper yellow; a sparse brown pattern, including a narrow, darker brown seam that extends from the stigma along the cord; a broader but paler crossband extending from the origin of Rs across the wing to the outer end of vein 2d A, vaguely and narrowly interrupted in cell M; a dark cloud at fork of M_{2+3}; veins yellow, darker in the clouded areas. Venation: R_{3+4} present as a distinct element, subequal to or longer than R_2; R_3 almost one-half the length of R_4.

Basal abdominal tergites black, the intermediate segments obscure yellow, the lateral margins broadly blackened; subterminal segments more uniformly darkened; hypopygium yellow. Male hypopygium (Plate 3, fig. 45) with the outer dististyle, *od*, having a slender, outer, curved spine and a more-elongate, stouter, inner arm, the median lobe with coarse setæ but without a spine, as in *signata;* inner dististyle a flattened pale blade, somewhat stouter than in *signata.*

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 27, 1931 (*Franck*). Allotype, female, altitude 9,000 feet, July 20, 1931. Paratype, male, with the allotype.

The only other described *Dasymallomyia* is the genotype, *D. signata* Brunetti, which has a wide range in the eastern Palæ-

arctic region, from the eastern Himalayas, through western China, to Formosa. It has been taken on Mount Omei but at lower altitudes than the present fly. *Dasymallomyia persignata* is most readily told from *signata* by the basal dark crossband of the wings, the dark cloud at fork of M_{2+3}, the longer vein R_3, and the slight distinctions in the male hypopygium.

ERIOPTERA (ILISIA) MEGAURA sp. nov. Plate 1, fig. 18; Plate 3, fig. 46.

General coloration gray, the præscutum with four darker plumbeous stripes; antennæ and legs black; knobs of halteres light yellow; wings obscure whitish, the base more yellow; veins black, coarse; anal veins gently diverging; male hypopygium large and powerful, the tergite extensive; outer dististyle a slender rod; inner style a powerful two-armed structure.

Male.—Length, about 5.5 millimeters; wing, 5.8 to 6.

Rostrum and palpi black. Antennæ black throughout; basal flagellar segments short-oval, the outer segments becoming slenderer and slightly more elongate outwardly; terminal segment shortest; verticils not or scarcely exceeding the segments in length. Head light gray; anterior vertex broad.

Mesonotum gray, the præscutum with four darker plumbeous stripes, the intermediate pair confluent in front of the level of the pseudosutural foveæ, the latter blackened; no brightening of the pretergal region. Pleura dark gray. Halteres with the stem slightly infuscated, the knobs light yellow. Legs with the coxæ gray; remainder of legs black. Wings (Plate 1, fig. 18) with the ground color obscure whitish, the anterior prearcular region and basal portions of the costal region more yellowish; stigma small, brown; axillary region and vein Cu vaguely infumed; veins coarse, black. Venation: Sc_1 ending just before level of R_2; Sc faint, about opposite one-third the length of Rs; vein 2d A nearly straight, gradually diverging from 1st A.

Abdomen black, the caudal margins of the segments more grayish; hypopygium black. Male hypopygium (Plate 3, fig. 46) large and powerful, the tergite, *9t*, extensive, arched. Outer dististyle, *od*, a slender, arcuated rod, its surface, except at extreme base, with microscopic appressed setulæ. Inner dististyle, *id*, large and bulky, the outer apical angle produced into a blunt blackened lobe, the inner angle extended into a compressed yellowish blade. Elements of the phallosome, *p*, forming flattened plates that are contiguous on the median line, the outer lateral angles produced into slender arms, the tips a trifle incurved, obtuse.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 9,000 feet, July 20, 1931 (*Franck*). Paratopotype, male.

The closest allied species is *Erioptera* (*Ilisia*) *bifurcata* Alexander (Japan), which has an almost identical appearance and venation but differs very notably in the structure of the male hypopygium, especially the elongate outer dististyle, the short, deeply furcate inner style, and the acicular gonapophyses. I consider that these two flies belong to *Ilisia*, where they are aberrant; they may be removed to some other group when allied forms are better known.

ERIOPTERA (TELENEURA) LEUCOPODA sp. nov. Plate 1, fig. 19.

General coloration of mesonotum dark brown; head and pronotum abruptly light yellow; antennæ black throughout; thoracic pleura brown, with a broad black longitudinal stripe; halteres brown; legs brown, the tarsi and tips of posterior tibiæ conspicuously whitened; wings broad, strongly infuscated; Rs fully one-half longer than R_{2+3+4}; abdomen black, the valves of the ovipositor abruptly horn yellow.

Female.—Length, about 4 millimeters; wing, 3.8.

Rostrum and palpi black. Antennæ black throughout; flagellar segments oval, becoming more elongate-oval outwardly; verticils conspicuous, fully one-half longer than the segments. Head clear light yellow, contrasting abruptly with the mesothorax.

Pronotum yellow, the anterior lateral pretergites light sulphur yellow. Mesonotum dark brown, the humeral portion of the præscutum a trifle brightened. Pleura brown, with a broad black longitudinal stripe, extending from the propleura to the abdomen, passing beneath the root of the haltere; dorsopleural region brownish yellow. Halteres brown, the base of stem narrowly pale. Legs with the coxæ and trochanters brown; femora dark brown; tibiæ brownish black, the tips of the fore and middle tibiæ very narrowly paler, of the posterior tibiæ broadly and conspicuously whitened; tarsi white, the basitarsi of fore and middle legs more or less infuscated on more than basal half. Wings (Plate 1, fig. 19) broad, strongly tinged with brown; veins and macrotrichia darker. Marginal fringes and trichia of veins long and conspicuous. Venation: Rs relatively long, fully one-half longer than R_{2+3+4}; R_2 subequal to R_{2+3}; m-cu close to fork of M; vein 2d A very gently sinuous.

Abdomen black. Ovipositor with the basal shields blackened, the valves abruptly horn yellow; cerci strongly upcurved to the acute tips, their margins smooth.

Habitat.—China (Szechwan).

Holotype, female, Mount Omei, altitude 7,000 feet, July 27, 1931 (*Franck*).

Erioptera (*Teleneura*) *leucopoda* is readily told from the other described species of the subgenus by the clear yellow head and conspicuously whitened feet. By my key to the species of *Teleneura*[3] the fly runs to *E.* (*T.*) *melanotænia* Alexander (Philippines), which differs conspicuously in the smaller size, differently colored head, and darkened feet.

ERIOPTERA (TELENEURA) LUTEICLAVATA sp. nov. Plate 3, fig. 47.

General coloration of mesonotum medium brown, the pleura yellow with a narrow brownish black longitudinal stripe; head light yellow; knobs of halteres light yellow; legs black; wings with a strong brownish tinge; abdominal sternites and hypopygium yellow; male hypopygium with both dististyles slender, the inner acutely pointed; gonapophyses appearing as simple blackened hooks.

Male.—Length, about 3.4 millimeters; wing, 4.2.

Rostrum and palpi black. Antennæ with the scape and enlarged pedicel black; flagellum dark brown; flagellar segments becoming slenderer and more attenuate outwardly; verticils long and conspicuous, much exceeding the segments. Head light yellow; vertex broad.

Pronotum yellow. Mesonotum medium brown; the anterior part of præscutum a little darker, the lateral præscutal margins testaceous-yellow. Pleura yellow, with a narrow but conspicuous brownish black longitudinal stripe extending from the fore coxæ caudad, passing just beneath the root of halteres; ventral sternopleurite infuscated. Halteres pale, the knobs, including the setæ, light yellow. Legs with the fore coxæ black, the remaining coxæ and all trochanters obscure yellow; femora and tibiæ black (a single leg, middle, remains, broken beyond tibia); no indication of brightening at apex of tibia, as in *leucopoda.* Wings broad, with a strong brown tinge, the prearcular and costal regions somewhat more yellowish; stigmal area slightly infuscated; veins pale, the macrotrichia somewhat darker. Venation: Vein 2d A gently sinuous, ending opposite m-cu.

[3] Philip. Journ. Sci. 46 (1931) 287.

Abdominal tergites brown, the sternites obscure yellow; hypopygium light yellow. Male hypopygium (Plate 3, fig. 47) with the outer dististyle, *od*, slender, the distal third blackened, the apex flattened and with parallel striæ. Inner dististyle, *id*, a little longer, appearing as a slender pale rod, the tip acutely pointed. Gonapophyses, *g*, appearing as simple curved hooks, the tips blackened, acutely pointed. Apex of ædeagus, *a*, very strongly curved.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

Erioptera (*Teleneura*) *luteiclavata* is most closely allied to *E.* (*T.*) *leucopoda* sp nov., from the same locality. The pale coloration of the thorax and abdomen, the yellow knobs of the halteres, and the unbrightened tips of the tibiæ, all preclude any reference of the present fly to *leucopoda*. It is unfortunate that all tarsi of the type are broken and it is thus impossible to determine whether or not the feet are whitened in any degree.

ORMOSIA PRÆCISA sp. nov. Plate 1, fig. 20; Plate 3, fig. 48.

General coloration of mesonotal præscutum and scutum reddish brown, the posterior sclerites and the pleura darker plumbeous brown; knobs of halteres light yellow; femora black, tibiæ and tarsi pale yellow; wings with a brownish tinge, sparsely variegated with small pale brown spots and whitish discal areas; veins R_3 and R_4 not curved strongly cephalad at tips; vein 2d A strongly sinuous; male hypopygium with three gonapophyses on either side.

Male.—Length, about 4 millimeters; wing, 4.5.

Head broken.

Pronotum dark brown. Mesonotal præscutum and scutum reddish brown, the posterior sclerites of mesonotum darker plumbeous brown, sparsely pruinose; pseudosutural foveæ and tuberculate pits brownish black. Pleura almost uniform plumbeous brown. Halteres dusky, the knobs light yellow. Legs with the small coxæ plumbeous-brown; trochanters brownish yellow; femora black; tibiæ and tarsi pale yellow. Wings (Plate 1, fig. 20) with a brownish tinge, sparsely variegated with pale brown spots and clouds at cord, fork of M_{2+3} and as small marginal clouds at ends of longitudinal veins; stigma dark brown, with a somewhat more yellowish area beyond it; disk of wing before cord, in outer ends of basal cells, and beyond cord in medial field, more whitish; veins pale, darker in

the clouded areas. Macrotrichia of cells abundant but pale. Venation: Sc_1 ending opposite R_2, Sc_2 about opposite midlength of Rs; R_2 just beyond fork of R_{3+4}, the latter veins not strongly curved cephalad at tips; m-cu just before fork of M; vein 2d A strongly sinuous.

Abdominal tergites dark brown, the sternites somewhat paler; hypopygium dark. Male hypopygium (Plate 3, fig. 48) with the outer dististyle, *od*, small, the surface of apical half with transverse rows of microscopic setæ. Inner dististyle, *id*, broad, the outer apical angle produced into a relatively slender lobe. Gonapophyses, *g*, appearing as three arms or branches on either side, these relatively slender, the stoutest and longest arm with about three subterminal teeth, its branch with a single small subterminal tooth.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

Ormosia præcisa is most nearly allied to a group of Japanese species, including *O. takahashii* Alexander, *O. takeuchii* Alexander, and *O. tokunagai* Alexander, differing especially in the structure of the gonapophyses of the male hypopygium.

MOLOPHILUS ARIEL sp. nov. Plate 1, fig. 21; Plate 3, fig. 49.

Belongs to the *gracilis* group and subgroup; general coloration black, sparsely pruinose to give a leaden appearance; antennæ (male) short; halteres black; male hypopygium with a single dististyle, this appearing as a stout rod that divides at apex into about eight slender branches.

Male.—Length, about 3 millimeters; wing, 3.8.

Rostrum and palpi black. Antennæ (male) short, black throughout; flagellar segments long-oval, the verticils exceeding the segments. Head dark gray.

Mesonotum and pleura entirely black, sparsely pruinose, to give a dark leaden appearance. Halteres black, the stem variegated by sparse yellow setæ. Legs brownish black. Wings (Plate 1, fig. 21) relatively narrow, strongly suffused with blackish; veins and macrotrichia black. Costal fringe and trichia of veins long and conspicuous. Venation: Sc_1 ending about opposite R_2; R_2 about in transverse alignment with r-m; vein 2d A ending opposite m-cu.

Abdomen, including hypopygium, black. Male hypopygium (Plate 3, fig. 49) with only two lobes of basistyle, *b*, developed, the dorsal, *db*, relatively small and slender, with long coarse

setæ distributed over its entire length; ventral lobe, *vb*, large, flattened, at apex further prolonged into a more glabrous outer lobe. A single dististyle, *d*, appearing as a stout rod that divides at apex into eight slender branches to produce a broom-like appearance, the most-basal branch shorter; at base of style a small fingerlike lobule that bears a few setæ. Ædeagus long, slender.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

The somewhat remarkable male hypopygium readily distinguishes this species from all others of the genus. *Molophilus albireo* Alexander (China) is very similar in its general appearance but has an entirely different genitalic structure.

MOLOPHILUS ANTARES sp. nov. Plate 1, fig. 22; Plate 3, fig. 50.

Belongs to the *gracilis* group and subgroup; mesonotal præscutum and scutum reddish brown, the posterior sclerites of notum and the pleura dark brown; legs and halteres dark brown; wings with a strong brown tinge; male hypopygium with the basistyle elongate, the apical lobes and dististyles relatively short when compared with the body of the style.

Male.—Length, about 3 millimeters; wing, 3.5.

Rostrum and palpi brownish black. Antennæ (male) short, brown throughout; flagellar segments cylindrical or nearly so, the verticils much longer than the segments. Head dark brown.

Mesonotal præscutum and scutum light reddish brown, the posterior sclerites of mesonotum and the pleura dark brown. Halteres infuscated, the base of stem restrictedly yellow. Legs with the coxæ and trochanters yellow; remainder of legs dark brown, the femoral bases restrictedly obscure yellow. Wings (Plate 1, fig. 22) with a strong brownish tinge, the prearcular and costal regions somewhat darker; veins pale, the darkest veins pale brown; macrotrichia dark brown. Venation: r_2 lying opposite or just beyond the level of r-m; vein 2d A relatively long and gently sinuous, ending shortly beyond m-cu.

Abdomen dark brown; hypopygium brownish yellow. Male hypopygium (Plate 3 fig. 50) with the basistyles, *b*, long and slender, the various apical lobes and the dististyles considerably shorter than the main body of the style; dorsal lobe of basistyle short, obtusely rounded at apex, the distal half without macrotrichia; ventral lobe, *vb*, short, with a dense group of coarse setæ at apex. Outer dististyle, *od*, irregular in outline, with

an outer blackened beak and an inner arm that bears two slender points, one being an acute spine. Inner dististyle, *id*, long, bent at near midlength, the apex microscopically roughened. Ædeagus, *a*, long and straight.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 7,000 feet, July 17, 1931 (*Franck*).

Molophilus antares is very different from the other regional species of the *gracilis* group in the structure of the male hypopygium, especially the great length of the basistyles in proportion to the apical lobes, and in the structure of the outer dististyle.

ILLUSTRATIONS

[Legend: *a*, ædeagus; *b*, basistyle; *d*, dististyle; *db*, dorsal lobe of basistyle; *g*, gonapophysis; *i*, interbase; *id*, inner dististyle; *od*, outer dististyle; *p*, phallosome; *s*, sternite; *t*, tergite; *vb*, ventral lobe of basistyle; *vd*, ventral dististyle.]

PLATE 1

FIG. 1. *Tipula* (*Trichotipula*) *polytricha* sp. nov., venation.
2. *Tipula mutiloides* sp. nov., venation.
3. *Macgregoromyia szechwanensis* sp. nov., venation.
4. *Macgregoromyia celestia* sp. nov., venation.
5. *Limonia* (*Limonia*) *francki* sp. nov., venation.
6. *Limonia* (*Limonia*) *laticellula* sp. nov., venation.
7. *Limonia* (*Limonia*) *bicornigera* sp. nov., venation.
8. *Limonia* (*Dicranomyia*) *sublimis* sp. nov., venation.
9. *Helius* (*Helius*) *infirmus* sp. nov., venation.
10. *Antocha* (*Antocha*) *constricta* sp. nov., venation.
11. *Antocha* (*Antocha*) *nigribasis* sp. nov., venation.
12. *Antocha* (*Antocha*) *bidens* sp. nov., venation.
13. *Adelphomyia latissima* sp. nov., venation.
14. *Phyllolabis pictivena* sp. nov., venation.
15. *Rhabdomastix* (*Palæogonomyia*) *omeina* sp. nov., venation.
16. *Gnophomyia collata* sp. nov., venation.
17. *Dasymallomyia persignata* sp. nov., venation.
18. *Erioptera* (*Ilisia*) *megaura* sp. nov., venation.
19. *Erioptera* (*Teleneura*) *leucopoda* sp. nov., venation.
20. *Ormosia præcisa* sp. nov., venation.
21. *Molophilus ariel* sp. nov., venation.
22. *Molophilus antares* sp. nov., venation.

PLATE 2

FIG. 23. *Tipula* (*Trichotipula*) *polytricha* sp. nov., male hypopygium, lateral aspect.
24. *Tipula* (*Trichotipula*) *polytricha* sp. nov., male hypopygium, ninth tergite.
25. *Tipula* (*Trichotipula*) *polytricha* sp. nov., male hypopygium, ninth sternite.
26. *Tipula* (*Trichotipula*) *polytricha* sp. nov., male hypopygium, styli.
27. *Tipula mutiloides* sp. nov., male hypopygium, lateral aspect.
28. *Tipula mutiloides* sp. nov., male hypopygium, ninth tergite.
29. *Tipula mutiloides* sp. nov., male hypopygium, styli.
30. *Tipula mutiloides* sp. nov., male hypopygium, eighth sternite.
31. *Macgregoromyia szechwanensis* sp. nov., basal five flagellar segments, male.
32. *Macgregoromyia szechwanensis* sp. nov., male hypopygium, dorsal.
33. *Macgregoromyia celestia* sp. nov., basal five flagellar segments, male.

FIG. 34. *Macgregoromyia celestia* sp. nov., male hypopygium, inner dististyle.
35. *Limonia* (*Limonia*) *francki* sp. nov., male hypopygium.
36. *Limonia* (*Limonia*) *bicornigera* sp. nov., male hypopygium.
37. *Limonia* (*Dicranomyia*) *sublimis* sp. nov., male hypopygium.

PLATE 3

FIG. 38. *Helius* (*Helius*) *infirmus* sp. nov., male hypopygium.
39. *Antocha* (*Antocha*) *constricta* sp. nov., male hypopygium.
40. *Antocha* (*Antocha*) *multidentata* sp. nov., male hypopygium.
41. *Antocha* (*Antocha*) *spiralis* sp. nov., male hypopygium.
42. *Antocha* (*Antocha*) *nigribasis* sp. nov., male hypopygium.
43. *Antocha* (*Antocha*) *bidens* sp. nov., male hypopygium.
44. *Adelphomyia latissima* sp. nov., male hypopygium.
45. *Dasymallomyia persignata* sp. nov., male hypopygium.
46. *Erioptera* (*Ilisia*) *megaura* sp. nov., male hypopygium.
47. *Erioptera* (*Teleneura*) *luteiclavata* sp. nov., male hypopygium.
48. *Ormosia præcisa* sp. nov., male hypopygium.
49. *Molophilus ariel* sp. nov., male hypopygium.
50. *Molophilus antares* sp. nov., male hypopygium.

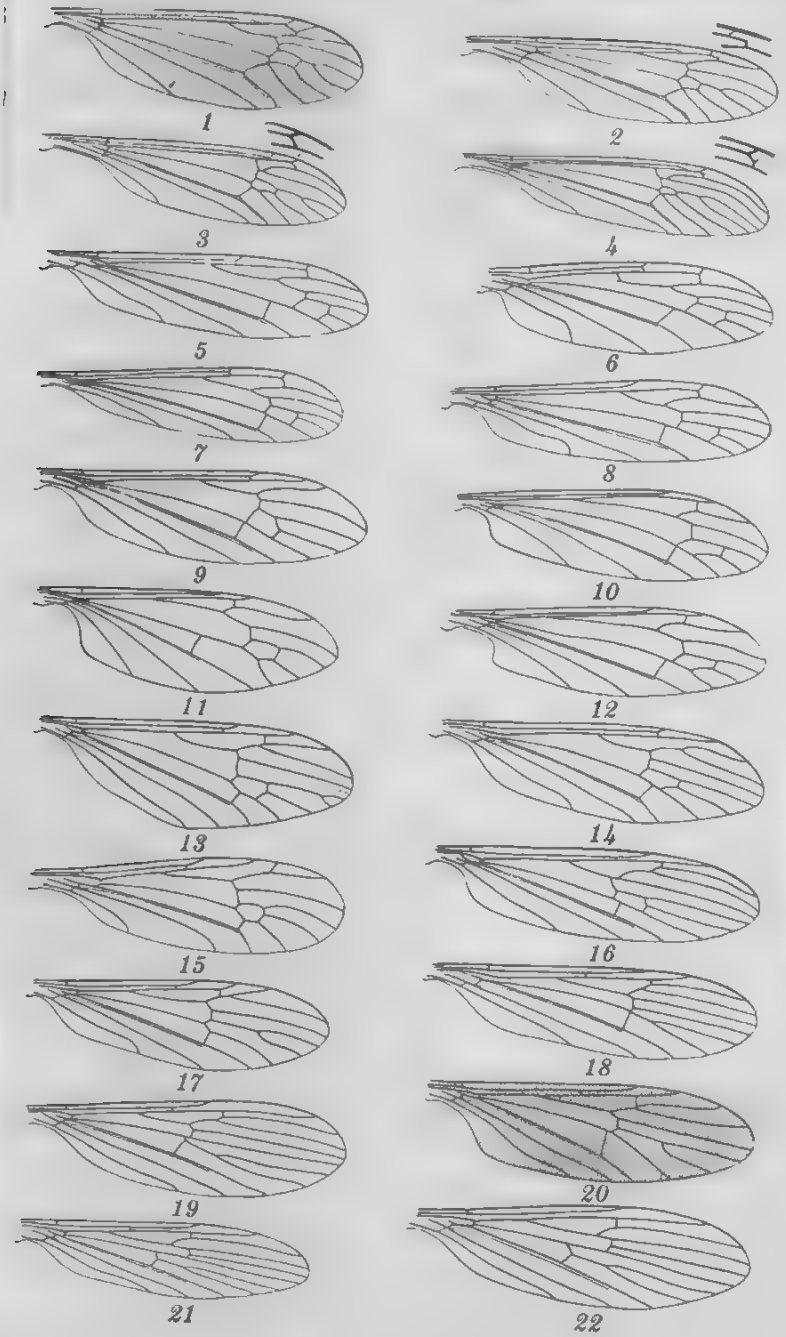
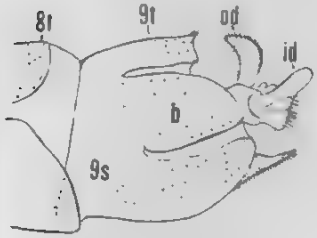
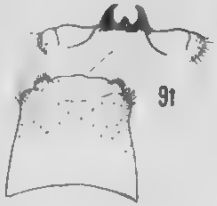


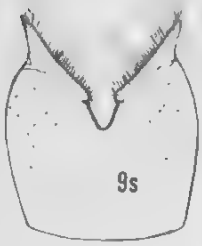
PLATE 1.



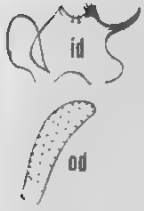
23



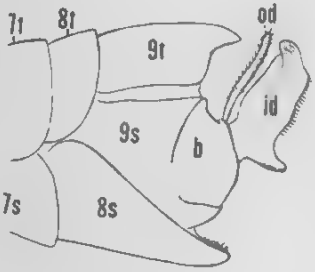
24



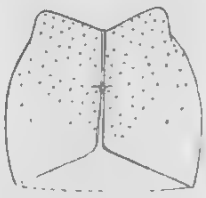
25



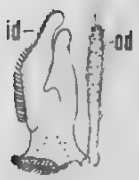
26



27



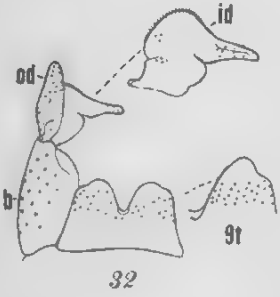
28



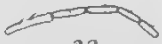
29

30

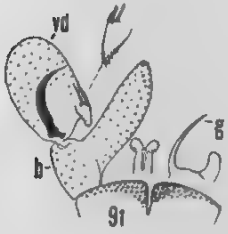

31



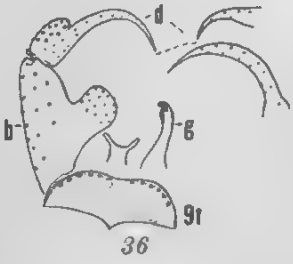
32


33

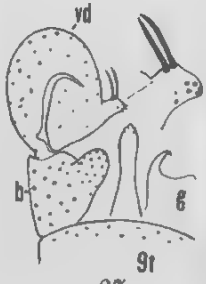
34



35



36



37

PLATE 2.

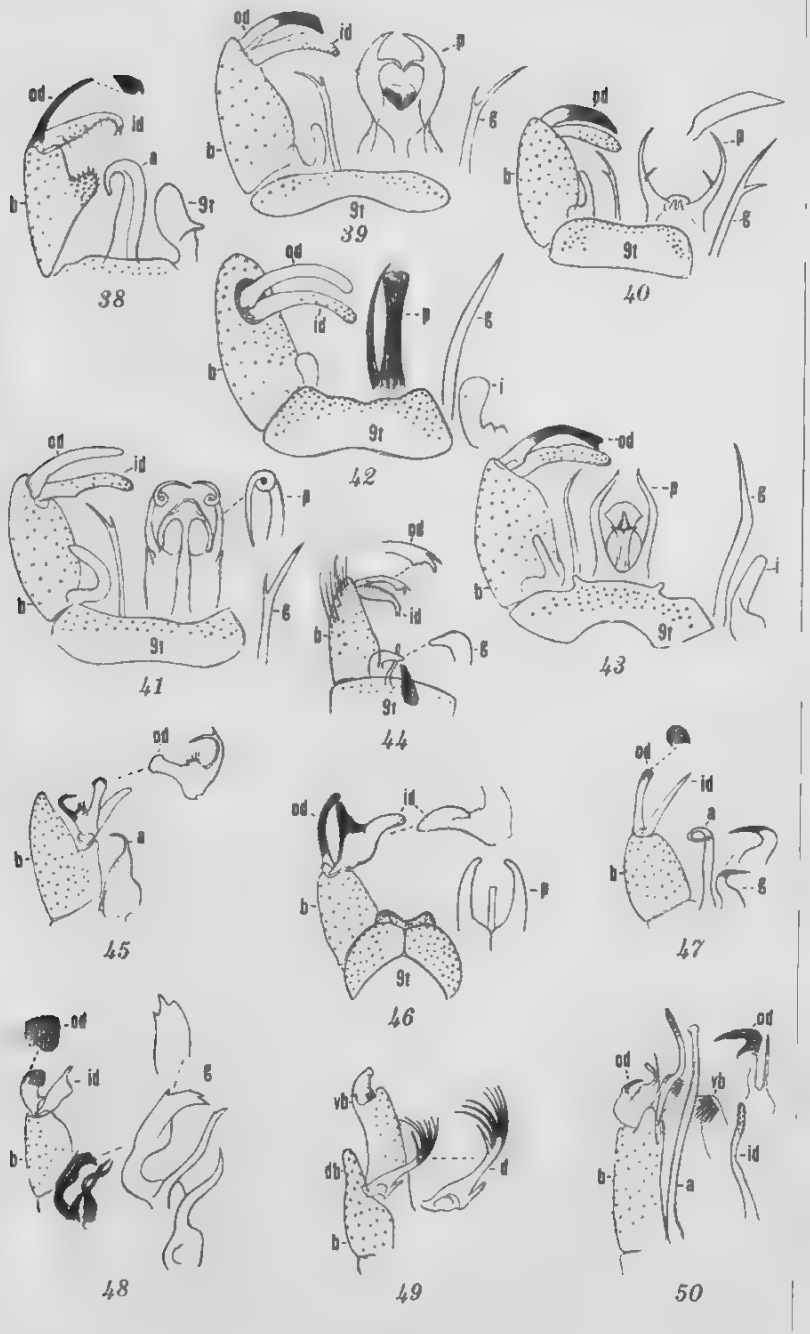


PLATE 3.

AN UNREPORTED FUNGOUS DISEASE OF THE PHILIPPINE MIGRATORY LOCUST [1]

By GAUDENCIO M. REYES

Of the Bureau of Science, Manila

FIVE PLATES

INTRODUCTION

Several parasitic fungi have been reported and described attacking insects, and in certain countries some of these fungi have been used with varying degrees of success in the control of destructive insect pests. Although these so-called entomogenous fungi help the farmer in his battles against economic insects, it would seem appropriate to cite the views of Charles,(6) as follows: "Although the results of studies have appeared from time to time, the subject (entomogenous fungi) as a whole has not been thoroughly investigated. That it offers much of purely scientific interest is unquestioned; what it still holds of potential economic value is yet to be discovered."

In the Philippines various fungi have been observed on a variety of insects, but little attempt has been made to study and describe them. In 1930, Celino(5) isolated a fungus from a dead coconut leaf-miner beetle, which was later identified by Dr. T. Petch(10) as *Beauveria bassiana* (Bals.) Vuill. Stevens,(15) in 1931, reported *Empusa grylli* (Fres.) Nowakowski on grasshoppers, *Cephalosporium crassum* Petch on *Pentalonia nigronervosa* Coq., and *Cordyceps podocreoides* von Höhnel on *Leucopholis irrorata* Chevrolat.

In September, 1929, at the Alabang locust laboratory and insectary of the former Bureau of Agriculture, a severe outbreak of a white fungus affecting the Philippine migratory locust, *Pachytylus migratorioides* Rch. and Frm. = *Locusta migratoria* ph. *migratorioides* Rch. and Frm.,(16) has attracted the writer's

[1] Submitted for publication December 1, 1931. The writer wishes to express his deep appreciation to Mr. S. S. Gonzales, in charge of locust research of the Bureau of Plant Industry at Alabang, Rizal Province, for furnishing the materials for this work. Thanks are also due Dr. C. J. Humphrey, mycologist, and Dr. T. G. Fajardo, of the botany division, Bureau of Science, for the critical reading of the manuscript.

attention. The infestation was so serious that nearly all the stock of locusts was lost. Because of the seriousness of this fungous disease on the migratory locust, a study of it might prove valuable in the locust problem in the Philippines. As locusts were not available in the locality or elsewhere during the course of these studies, field experiments were abandoned. The present paper, therefore, only deals with the description of the disease and certain studies on the pathogenicity, morphology, behavior of the fungus in artificial media, and persistence of the fungus in culture.

DESCRIPTION OF THE DISEASE

The early symptoms of the insect which becomes infected from artificial inoculation is the loss of appetite, apparent weakness and sluggishness, loss of the power of locomotion as if the muscles were paralyzed, and sooner or later death. The infected insect becomes somewhat darker than its normal color and upon dissection, fungous threads were found in the air cavities of the thorax (Plate 5), and in the thoracic and abdominal tissues. Tufts of mycelia can be seen coming out from certain parts of the body and spreading over the chitinous integument. The most striking external appearance of the disease, however, is the presence of cottony-white, floccose growth, protruding from the junctures of the body segments and the joints of the legs (Plate 5). Later these mycelial white masses sporulate heavily, and the color changes from white to ivory or creamy white. The insect afterwards becomes mummified (Plate 1, fig. 1) and the fungus can be seen practically covering the entire carcass.

THE CAUSAL FUNGUS

ISOLATION

The fungus was readily isolated by the spore dilution plate method (Plate 1, fig. 2). With this method a small amount of the fungus containing spores was first removed from fresh specimens and suspended in sterile distilled water. This suspension was further diluted by means of loop transfers into other tubes of sterile water. From the three dilutions, 1 to 3 loopfulls were transferred into tubes of melted potato glucose agar which had been cooled to 40° C., and vigorously shaken before they were poured into sterile Petri dishes. After two to three days incubation at room temperature, identical single spore colonies developing in the second and third plates of the

series were isolated, preferably from the latter (Plate 1, fig. 2), and transferred into suitable media. The colonies obtained were essentially the same as the original material. All cultures used in subsequent studies of the fungus mentioned below were derived from single spore isolation.

PATHOGENICITY

Inoculations with the fungus obtained from single spore colonies proved beyond doubt the parasitic potentiality of the organism. In these experiments, healthy locusts reared from eggs were incarcerated in sterilized, relatively small wire cages and were supplied with abundant fresh, green grass planted in small beakers containing moist, sterilized sand. These were kept in the laboratory at temperatures ranging from 28° to 32° C. or over. The spores were applied on the insects either by spreading under the rudimentary wings or on the thoracic side, or by atomizing the insects direct on the leaves they ate with spores in sterile water suspension. For controls, the locusts were either left without treatment or sprayed with plain sterile water, depending on the method used.

The effect of infection was invariably fatal, death occurring after one to six days, the newly molted insects falling easy victims to the disease. The insects in the control remained still alive when examined after fifteen days. Reisolations proved positive. There was actually no substantial difference between the course of the disease in nature and in the laboratory.

The fungus readily attacks the locust in all its stages (Plate 5), causing from 60 to 100 per cent infection, and present results of carefully conducted experiments in October and November, 1929, seem to indicate that, as a rule, the younger the locust the more easily it succumbs, probably because the young are comparatively weaker, their cuticle being softer and thinner, and because of the comparative absence of wings or body protection. It was likewise found that those that molted immediately after the spores were applied usually escaped death. Following the suggestion of Howard,(9) it was further observed that the inoculations made in the afternoon gave better results, and a prolonged period of warm, humid weather favored the development of the fungus greatly. For lack of materials, no inoculation was attempted to infect egg pods.

In view of the fact that representatives of the genus *Beauveria* have been found to occur and infect different kinds of insects, trials were made to communicate artificially the locust fungus

on the adult coconut leaf miners. In a series of tests conducted in large glass jars in the greenhouse and in relatively large wire cages in the open, its parasitic nature has been proven definitely on the coconut leaf-miner beetles, and the results obtained are printed in this issue.

MORPHOLOGY

The mycelium is septate, cottony-white, floccose, and branches out usually at right angles (Plate 3, fig. 3). The hyphæ are often connected by H-shaped unions or anastomosing branches (Plate 3, fig. 4). The mycelium is hyaline and granular when young, but later some may become somewhat vacuolated. In culture abundant conidia are produced from a sporiferous hymenial layer. A secondary crop of mycelia later appears on the surface from which a layer of conidia are again produced.

The conidia are minute, hyaline, smooth, single-celled (Plate 3, fig. 1), borne on slender flexuous sterigmata at the apex of each phialide (Plate 4, fig. 2). They are globose, rarely globoid (Plate 3, fig. 1, and Plate 4, fig. 3), having an average diameter of 2.17 microns, based on 100 measurements. Myriads of conidia are produced in loose globose heads either on the main hyphal branches or on short lateral ones. The conidia germinate readily in water, swelling overnight, and produce germ tubes which are constricted at the base (Plate 3, fig. 2). As the germ tubes grow, they in turn produce normal heads of globose conidia.

The conidiophores are short and simple and have a bottle- or flask-shaped "phialide" appearance. Each phialide bears one or sometimes two filiform, usually flexuous sterigmata (Plate 4) from which conidia are borne in a verticillate fashion. With the use of a filar ocular micrometer, the average diameter of the sterigmata is found to be 0.59 micron. The morphological features of the phialides, of the threadlike flexuous sterigmata, and of the successive production of globose conidia answer closely the essential characters of *Beauveria globulifera* or *Sporotrichum globuliferum* described by Dufrénoy(7) and Petch.(11)

BEHAVIOR OF THE FUNGUS ON DIFFERENT MEDIA

The fungus is readily cultivated on various media containing vegetable and animal substances. The type of colonies produced varied only slightly, but the density of growth and pigment production differed with the media used and with the age of

the culture. Good growths were obtained from potato-dextrose agar, potato cylinders, steamed rice, cornmeal, cornmeal-beef agar and beef-malt agar, but a rather scanty growth was obtained on sterilized string beans, sterilized locust, sterilized coconut leaf-miner beetles, or on decoctions made from these insects. The following media were tested and the characteristic growths were noted.

Potato-dextrose agar.—The fungus grew on the surface bulging at the point of inoculation. Zonations were produced sloping rather indistinctly towards the margin of the colony. Later the white surface growth turned Ivory Yellow [2] and the zonation became barely conspicuous because of the formation of a sporogenous layer. As the medium dried up, a secondary, aërial, mycelial growth was produced, which later became inconspicuous by the subsequent formation of abundant spores (Plate 2, fig. 2). Through repeated subcultures the zonation became less distinct or entirely absent.

Potato cylinders.—A cottony-white, elevated, floccose growth (Plate 2, fig. 1) was obtained from this medium. A rather indistinct zonation of the colonies was observed. The medium turned Purplish Vinaceous, near the vicinity of the colonies corroborating the findings of Pettit, (12) and the fungus on the slant surface was Ivory Yellow. As the growth advanced, the pigment on the medium was obscured by the growth of mycelium, and the colonies became convoluted. A secondary growth of mycelium also took place and a few coremialike structures starting as small, buttonlike outgrowths on the surface have been observed. In subsequent cultures, however, these structures were hardly noticed.

Steamed rice.—The fungus grew rather luxuriantly on this medium, producing an abundant aërial, white, floccose growth (Plate 2, fig. 1), especially on steamed glutinous rice. On aging, the fungus became Ivory Yellow to Cream Color due to the formation of numerous conidia. A secondary mycelial growth was developed on different portions of the medium, occasionally clogging the tube. Later this gave rise to a few coremialike growths ranging from 9 to 11 millimeters in height, as the medium lost much of its moisture (Plate 2, fig. 2). In later subcultures, however, very few or none of these growths were formed. No color reaction was observed.

[2] Capitalized color names are those of Ridgway's Color Standards and Color Nomenclature. Washington, D. C. (1912).

Cornmeal.—The fungus grew fairly well on cornmeal. As the growth thickened the fungus became tinged with Ivory Yellow and small scattered masses of cottony-white growth developed on the surface of the medium. The colony later became Cream Color with a faint purplish pigment produced in the immediate vicinity of the colonies.

Cornmeal-beef agar.—Growth was rapid, and the mycelium penetrated the substratum showing submerged development. Ivory Yellow color was produced on the sporiferous surface. A diffused Vinaceous Fawn color reaction took place in the substratum, which was not observed on other media.

Beef-malt agar.—Growth was very fast; the colony was dense, circular, and bulging aërially. In advanced stage of growth, zonations became somewhat distinct at the edge of the cultures. Ivory Yellow color appeared in the center of the colony as abundant spores were produced. No secondary crop of mycelium was noticed.

Potato agar.—The fungus growth was slow and scanty. Submerged growth of the mycelium was observed as in cornmeal-beef agar, but the conidia were produced on the surface, showing a somewhat powdery or chalky appearance. Later an Ivory Yellow tint was observed on the surface.

Sterilized string beans.—The fungus grew rather scantily but aërial mycelium was produced. The surface of the colony was white at first but later turned to Cartridge Buff as powdery masses of spores were produced. The beans were considerably consumed and became more or less transparent, leaving practically only the epidermal tissue.

From observations made of the growth characteristics of the fungus on the above-mentioned media, it is apparent that an elevated, cottony, floccose growth was distinctly and consistently shown on potato cylinders, steamed rice (glutinous or nonglutinous), potato-dextrose agar, and cornmeal, while a somewhat powdery or chalky type of growth was observed on cornmeal-beef agar, potato agar, beef-malt agar, and sterilized string beans.

IDENTITY AND SYNONYMY OF THE FUNGUS

The fungus under consideration appears to be identical in gross morphological and physiological characteristics with *Sporotrichum globuliferum* Speg. or *Beauveria globulifera* (Speg.) Pic., described by Saccardo,(13) Pettit,(12) Dufrénoy,(7) Petch,(11) and others. This fungus is characterized by the white, cottony,

floccose growth. The conidia are globose, measuring on an average 2.17 μ, which approximates the recorded spore measurements of Spegazzini, as cited by Saccardo,(13) and Pettit.(12) For purposes of verification, cultures of the fungus and a photograph of infected locusts were sent to Dr. R. Thaxter, of Harvard University, who in a letter to the writer, dated July 20, 1930, states that he has no material for comparison, but "it seems exactly similar to the ubiquitous and more or less omnivorous *Beauveria globulifera.*" The genus *Beauveria*, to which this species belongs, is an imperfect fungus of the tribe Verticillieæ. Various confusion in terminology has been made of representatives of the genus. Petch's treatise on *Beauveria*(11) states that the European writers have referred to members of this genus as *Botrytis* while the American mycologists placed them under *Sporotrichum* Link. In view of this confusion, the same author states that Vuillemin, in 1912, created a new generic name, *Beauveria*. As cited by Petch,(11) in 1914, Picard transferred Spegazzini's species, *Sporotrichum globuliferum*, to Vuillemin's genus as *Beauveria globulifera*. The writer, therefore, uses *Beauveria globulifera* for the parasite found on the Philippine migratory locust. Although the name *S. globuliferum* has been relegated to its synonymous binomial, *Beauveria globulifera*, some authors continue to refer to the fungus as originally described, and the names are used interchangeably.

DISTRIBUTION AND HOST RANGE

Among the entomogenous fungi, the genus *Sporotrichum*, or *Beauveria*, is known to be fairly widespread. According to Castellani,(4) the genus *Sporotrichum* has a large number of species found the world-over. Saccardo(13) and Petch,(11) citing the work of Spegazzini, stated that *Sporotrichum globuliferum* was found on Coleoptera, *Monocrepidium* sp., and *Naupactus xanthographus* in the Argentine, and subsequently on a *Gargaphia* (Hemiptera), also from the same country. Bruner(3) found *S. globuliferum* in 1897 in the Argentine and he, as well as Billings and Glenn,(2) and Headlee and McColloch,(8) found it parasitic on the chinch bug, *Blissus leucopterus* Say, in the United States. Bruner(3) and Headlee and McColloch(8) observed it on other insects. Bruner(3) further reported that a large migratory locust, *Schistocerca paranensis* Burm., was attacked by a species of *Sporotrichum*, the action of which is similar to that of *S. globuliferum* on the chinch bug. Howard,(9) in 1901, reported the successful infection of destruc-

tive grasshoppers in Colorado by a species of *Sporotrichum*. Petch(11) states that the parasitism of a given species of *Beauveria* is probably not restricted to one species of insect but to other groups of insects as well. Benois,(1) in 1928, reported the occurrence of five species of *Beauveria* on Acrididæ in European and Asiatic Russia. In 1929, Seymour(14) listed *S. globuliferum* as occurring on a wide variety of insects, including an acridid, *Melanoplus spretus* Uhl., and gave reference of its synonymy to *Beauveria globulifera* on certain species of *Cicada*, *Blissus*, and *Diabrotica*.

In the Philippines, the distribution of *B. globulifera* on the migratory locust is not definitely known, and as far as the writer is aware, no report of its occurrence on locust or other insect has been made.

VIABILITY OF THE FUNGUS

In series of experiments it was found that *B. globulifera* could persist in culture media under laboratory conditions and in the ice box for some time. Colonies kept at 28 to 32° C. for two hundred ninety-five days on steamed rice were still viable when transfers were made into fresh potato glucose agar. After four hundred forty days, colonies grown on steamed nonglutinous rice were already dead. The culture on sterilized locust was found dead at the end of two hundred twenty-five days. Colonies kept in potato dextrose agar, and steamed rice (nonglutinous and glutinous) at 13 to 15° C. were found viable at the end of two hundred ninety-five days. On glutinous rice cultures three hundred twenty days old, it was found still living. The summary of results is shown in Table 1.

TABLE 1.—*Viability of the fungus in culture media.*

Medium used as substrate.	Place of storage.	Temperature.	Age of culture.	Result.
		°C.	*Days.*	
Potato-dextrose agar	Room	28–32	225	Viable.
Do	do	28–32	295	Do.
Sterilized locust	do	28–32	225	Dead.
Steamed rice, glutinous	do	28–32	295	Viable.
Steamed rice, nonglutinous	do	28–32	295	Do.
Do	do	28–32	440	Dead.
Potato-dextrose agar	Ice box	13–15	225	Viable.
Do	do	13–15	295	Do.
Steamed rice, nonglutinous	do	13–15	295	Do.
Steamed rice, glutinous	do	13–15	295	Do.
Do	do	13–15	320	Do.

There are not enough comparative tests made of cultures kept in the two conditions, but it appears from the meager record obtained that the cultures kept in the ice box would live longer than those kept in the laboratory. Steamed glutinous rice seemed to have maintained the growth better and longer than the other kinds of media, probably because of its adhesiveness and capacity to retain moisture for a considerable period. These tests were not sufficiently extended or carried out on a large enough scale to warrant a definite conclusion, however.

SUMMARY

1. An entomogenous fungus attacking the Philippine migratory locust, *Pachytylus migratorioides* Rch. and Frm., is here reported for the first time in the Philippines.

2. The disease in an early stage can be readily recognized by the presence of white, cottony, floccose mycelial growths protruding from the junctures of body segments and joints of the legs, which in an advanced stage become sporiferous, the color changing to ivory or creamy white.

3. The fungus was isolated in pure culture and proved highly pathogenic on the Philippine migratory locust. The younger stages of the locust and those newly molted succumbed quicker than the adults.

4. The fungus has been cultivated on various artificial media containing a plant or an animal substance, or both, and spores were produced in enormous numbers.

5. The morphological and physiological characteristics of the fungus are identical with those of *Beauveria globulifera* (Speg.) Pic., formerly known as *Sporotrichum globuliferum* Speg., which attacks the chinch bug, *Blissus leucopterus* Say, in the United States. The fungus is characterized by having globose conidia and by the cottony-white, elevated, floccose growth in culture media.

6. On artificial media the fungus was still viable after two hundred ninety-five days at room temperature (28 to 32° C.), and in the ice box (13 to 15° C.) after three hundred twenty days. Steamed glutinous rice seemed to have maintained the growth better and longer than the other kinds of media used.

LITERATURE CITED

1. BENOIS, K. A. Fungal diseases of locusts. Summary of literature and report. Pamphlet issued by the mycology and phytopathological laboratory of A. A. Jaczewsky. State Institute of Experimental Agriculture, Leningrad (1928) 49, 18 figs. (In Russian.) *Cited in* Rev. App. Myc. 8 (1929) 379–380.

2. BILLINGS, F. H., and P. A. GLENN. Results of the artificial use of the white-fungus disease in Kansas. U. S. Dept. Agr. Bur. Ent. Bull. 107 (1911).
3. BRUNER, LAWRENCE. Killing destructive locusts with fungous diseases. U. S. Dept. Agr. Bull. 38 n. s. (1902).
4. CASTELLANI, ALDO. Fungi and fungous diseases. (Reprinted from the Arch. Dermatol. and Syphilol.) Am. Med. Assn., Chicago (1927–1928) 203 pp.
5. CELINO, M. S. Note: A fungous disease of the coconut leaf miner (Promecotheca cumingii Baly). Philip. Agr. 19 (1930) 253.
6. CHARLES, V. K. Entomogenous fungi attack and destroy many harmful insects. U. S. Dept. Agr. Yearbook (1931) 211–214, fig. 49.
7. DUFRÉNOY, JEAN. Metaphanic and progressive variation in Beauveria: Its phyletic significance. Mycologia 11 (1919) 276–277.
8. HEADLEE, T. J., and J. W. MCCOLLOCH. The chinch bug. Kansas Agr. Exp. Sta. Bull. 191 (1913) 287–353.
9. HOWARD, L. O. Ensayos sobre las enfermedades fungosas de las langostas. (Traducción por Sixto S. Sandejas de la edición Ingles.) Boletín del Agricultor No. 6. Segunda Edición (1904).
10. OCFEMIA, G. O. Notes on some economic plant diseases new in the Philippine Islands: II. (Fungous diseases of important insects.) Philip. Agr. 19 (1931) 581–589.
11. PETCH, T. Studies in entomogenous fungi VII. Notes on Beauveria. Trans. Brit. Mycol. Soc. 10 (1926) 244–271, 1 fig.
12. PETTIT, R. H. Studies in artificial cultures of entomogenous fungi. Cornell Univ. Agr. Exp. Sta. Bull. 97 (1895).
13. SACCARDO, P. A. Sylloge Fungorum. R. Friedlander and Sohn, Berlin 4 (1886) 96–113.
14. SEYMOUR, A. B. Host index of the fungi of North America. Harvard University Press. Cambridge, Mass. (1929) xiii + 732.
15. STEVENS, F. L. New or noteworthy Philippine fungi. Philip. Agr. 20 (1931) 87–91.
16. UVAROV, B. P. A revision of the genus Locusta, L. (= *Pachytylus*, Fieb.) with a new theory as to the periodicity and migrations of locusts. Bull. Ent. Res. 12 (1921) 135–163.

ILLUSTRATIONS

[Photographs were made by G. Panlilio and V. Ferrer, of the Bureau of Science.]

PLATE 1

FIG. 1. Two mummified specimens of the Philippine migratory locust, *Pachytylus migratorioides* Rch. and Frm., practically covered with the white fungus *Beauveria globulifera* (Speg.) Pic., from which the culture was obtained. Note that the fungus is densely crowded over certain areas, as between segments of the body. About 1.2 times natural size.

2. Spore dilution of the fungus, 6 days old, on potato-dextrose agar + 1 F. S. Note the uniformity of the elevated, cottony, floccose mycelial growth of the colonies. × 1.

PLATE 2

FIG. 1. Cultures of *Beauveria globulifera* on two potato slants and two steamed-rice slants, 46 days old, showing aërial, cottony-white, floccose growths. About two-thirds natural size.

2. The tube to the left is a 3.5-month-old culture of the fungus on potato-dextrose agar slant, showing typical, raised, white growth of *B. globulifera*. The other two tubes in the middle and to the right, respectively, are drying cultures on potato cylinder and steamed rice, showing a few coremialike structures which are more visible in tube on the extreme right.

PLATE 3

[Camera-lucida drawings, × 1900.]

FIG. 1. A group of globose spores obtained from an old culture of the organism.

2. Germinating conidia kept overnight in water. Note the swelling of the conidia and constriction at the base of the germ tubes.

3. Hyphal threads showing septation and lateral branching.

4. Hyphæ showing considerable anastomosing.

PLATE 4

FIG. 1. Camera lucida drawings. *a*, Conidia abstricted singly from the apices of bottle-shaped, paired phialides. Note the end of the mycelial hypha budding an isolated conidium. Drawn from mounts obtained from 9-day-old colonies. × 1900. *b*, A camera lucida drawing, showing subsequent swelling of the phialides and the production of typical, slender, zigzag *Beauveria* sterigmata. Note that one of the phialides has two sterigmata. Drawn from 10-day-old colonies. × 1900.

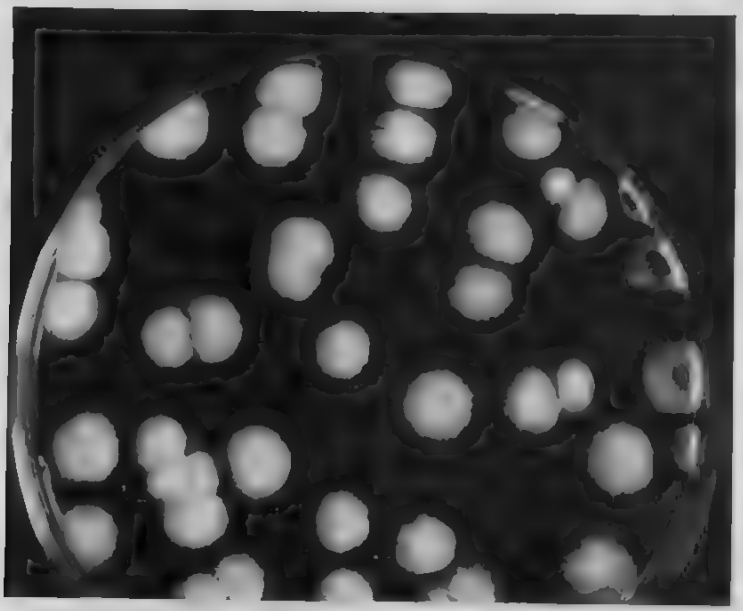
FIG. 2. A vegetative hypha bearing flask-shaped phialides on the swollen phialidiferous part of the hypha. Note the conidia borne on short pedicels in a whorl-like arrangement, one below the other, on the flexuous sterigmata. Drawn with the aid of camera lucida. × 1900.

3. A photomicrograph of *Beauveria globulifera* showing zigzag sterigmata (about the center), dispersed spores, and a conidial head at the bottom (somewhat dimmed by a water bubble). × 900.

PLATE 5

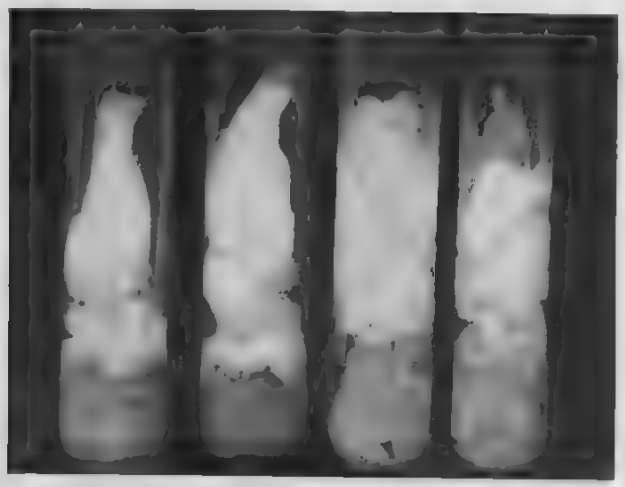
Nymphs and adult Philippine migratory locusts artificially infected with *Beauveria globulifera*. Note the vigorous development of an identical fungus on the chitinous integument, emanating densely from the intersegmental membranes. Note also the fungous growths in the air cavities, and the invaded tissues in the thoracic and abdominal regions of the dissected adult insect.

1

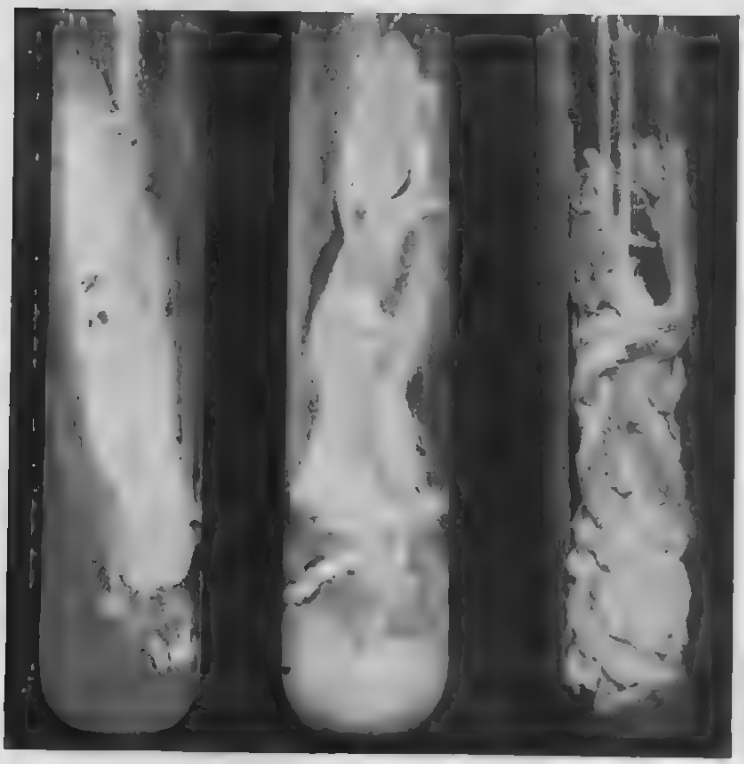


2

PLATE 1.



1



2

PLATE 2.

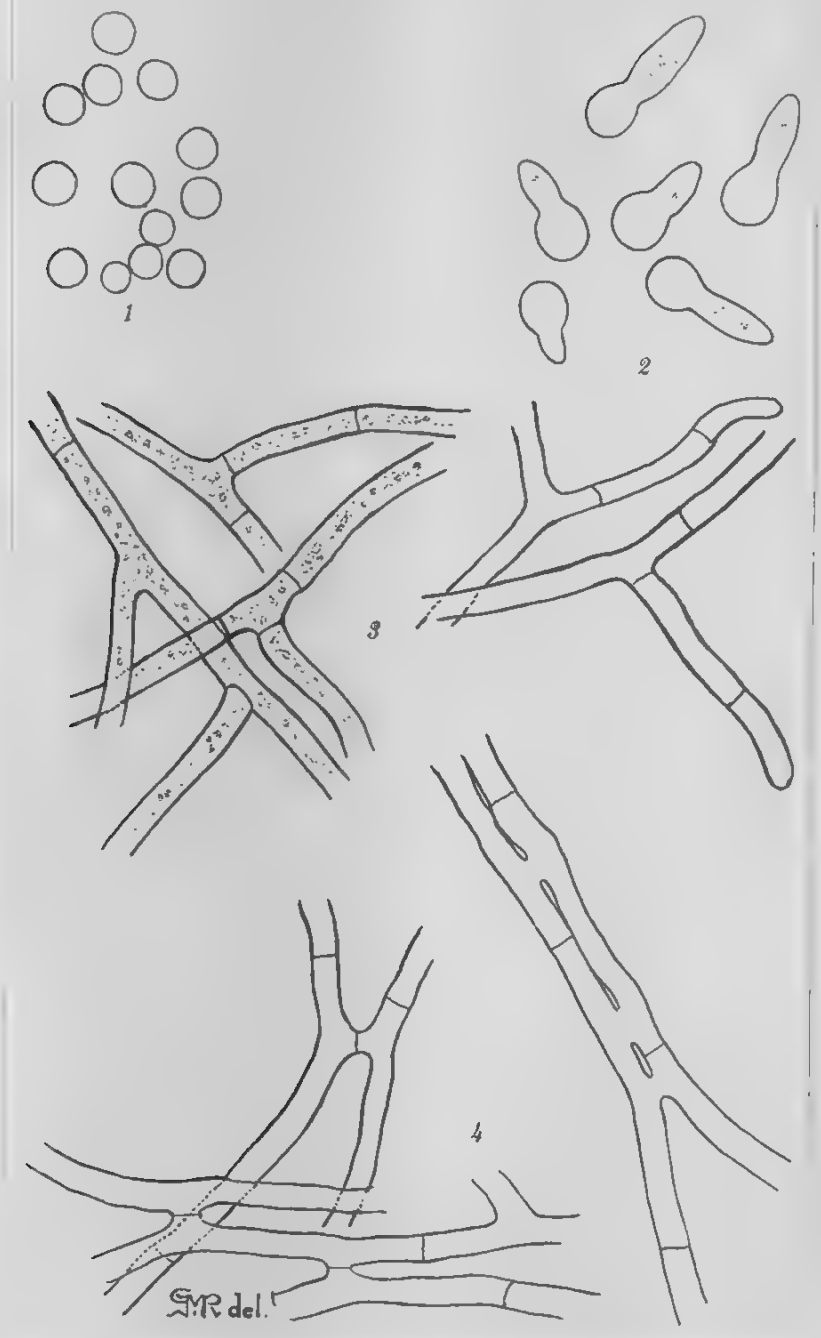
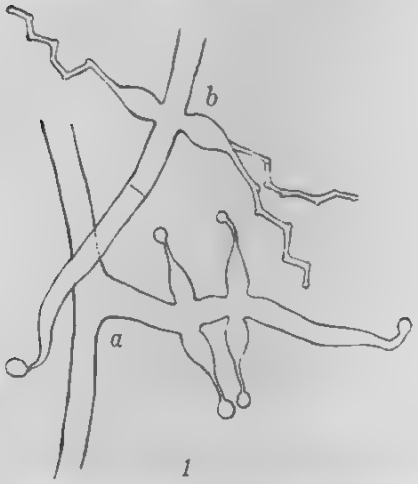
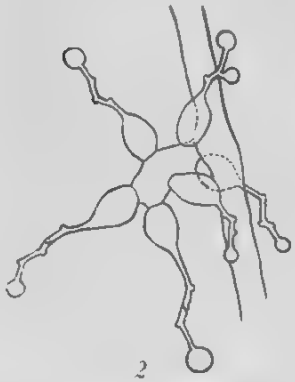


PLATE 3.

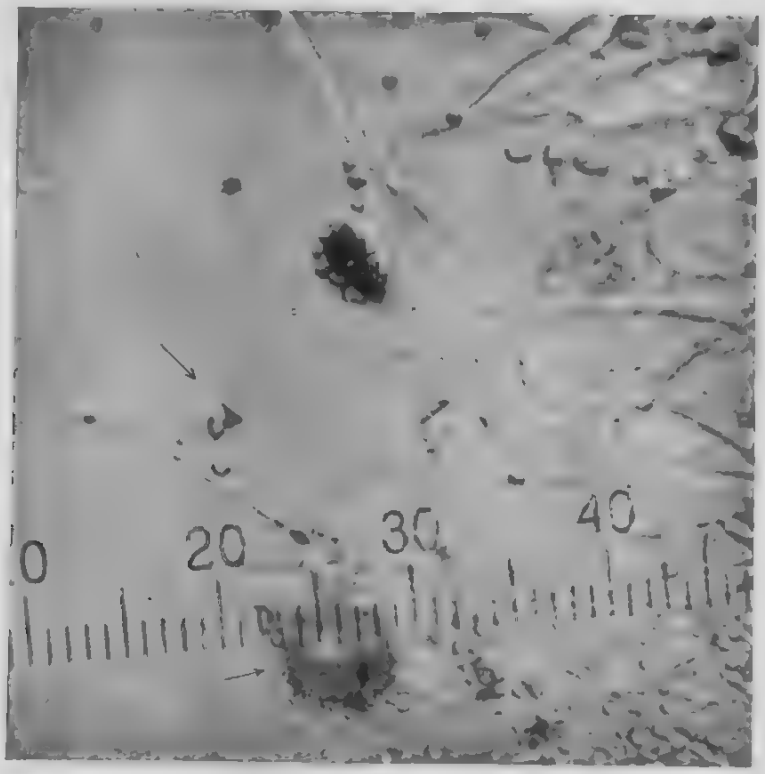


1



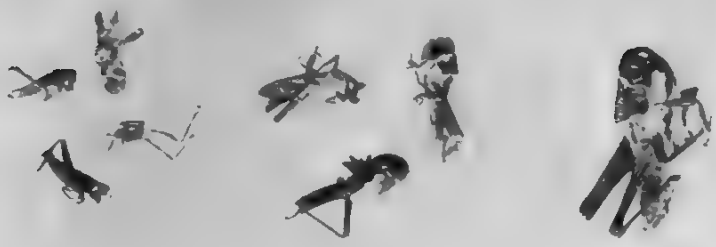
2

SMR del

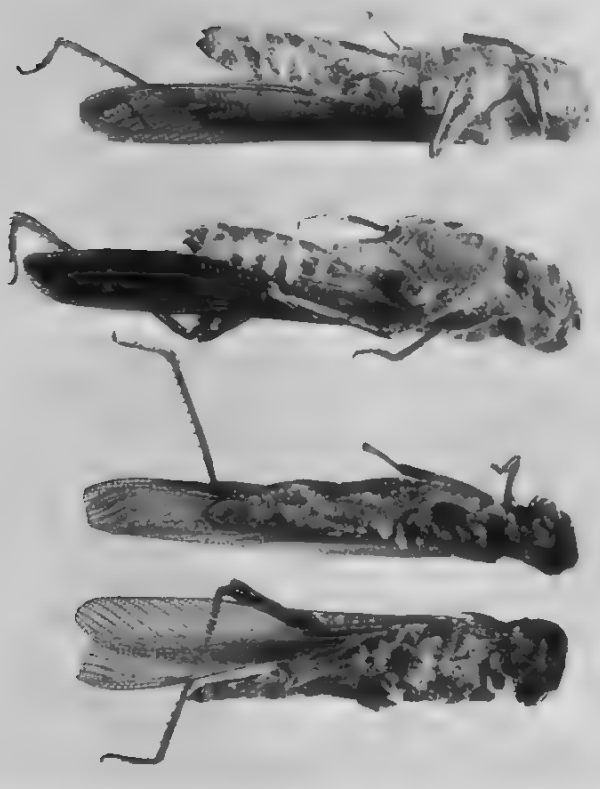


3

PLATE 4.



1



2

PLATE 5.

ARTIFICIAL INFECTION OF THE COCONUT LEAF MINER WITH BEAUVERIA GLOBULIFERA (SPEGAZZINI) PICARD[1]

By GAUDENCIO M. REYES

Of the Bureau of Science, Manila

FIVE PLATES

INTRODUCTION

The outbreak of an unusually heavy infestation of the coconut leaf miner, *Promecotheca cumingi* Baly,(19) in the Philippines in the latter part of 1929, afforded a splendid opportunity for investigations along biological lines. Six species of hymenopterous parasites have been discovered by entomologists of the Philippine Bureau of Plant Industry and these played a prominent rôle in the control of the coconut leaf miner.(1, 19) No less interesting perhaps are the fungous diseases that have been observed and cultivated. Celino has reported the occurrence of a fungus isolated from the coconut leaf-miner beetle,(5) which has been determined by Dr. T. Petch(15) as *Beauveria bassiana* (Bals.) Vuill.

The object of the present work was to study, by means of artificial cultures, the practicability of introducing the fungus *Beauveria globulifera* (Speg.) Pic., formerly known as *Sporotrichum globuliferum* Speg., isolated from the Philippine migratory locust, *Pachytylus migratorioides* Rch. and Frm.,(18) into the leaf-miner-infested regions. The writer's first efforts were directed toward determining the best method of dissemination. Unfortunately, on account of the limited time and facilities afforded, the writer had no opportunity to extend the investigation into the field on a large scale. However, certain significant features of value have finally been evolved, these being the results partly of outdoor experiments and observations.

[1] Submitted for publication December 1, 1931. The writer wishes to express his gratefulness to Dr. T. G. Fajardo, of the division of botany, Bureau of Science, for certain suggestions and criticisms in the preparation of the manuscript, and to Dr. C. J. Humphrey, mycologist of the same bureau, for reading it.

DISTRIBUTION OF THE FUNGUS, OTHER HOSTS, AND ECONOMIC IMPORTANCE

As recorded in scattered literature, *Beauveria globulifera* occurs on various important insects in widely separated geographical regions. Available reports of the fungus show that it occurs definitely in the United States(4, 9) on the chinch bug, *Blissus leucopterus* Say, and, according to Headlee and McColloch,(9) on other insects of more or less economic importance. Dozier(7) found it on the larvæ of the codling moth, *Carpocapsa pomonella*, on apples in Delaware. Other reports of the fungus and the insects it attacks show that it also occurs in France,(3, 11, 12) in the Argentine,(17) and in the West Indies.(2, 14) There is little doubt of its occurrence in other foreign countries, especially in tropical and subtropical regions.

Authorities seem to disagree as to the efficacy or possibility of introducing or disseminating fungous parasites among insects; some think it utterly impracticable, while others believe it of considerable economic importance, at times exerting a great influence, while still others believe that the subject has not been thoroughly studied and its potential importance has not been unravelled as yet. For many years certain destructive insect pests have been fought more or less successfully by the artificial propagation and introduction of different kinds of entomogenous fungi and among these insect-destroying fungi *Sporotrichum globuliferum*, or *Beauveria globulifera*, plays an important part. In general, parasitic fungi help check the multiplication of injurious insects and help man in his struggles against these pests. Petch, cited by Martin,(13) states that the problem now confronting investigators of entomogenous fungi centers on how to be able to induce an epidemic upon insects at a time when an epidemic would not take place naturally.

As far as the writer is aware, *Beauveria globulifera* has not been reported as occurring on the coconut leaf miner, *Promecotheca cumingi* Baly, nor has there been an attempt to infect artificially this economic insect with this fungus. Celino,(5) however, has succeeded in artificially infecting adult coconut leaf miners with a closely allied fungus, which was later identified by Doctor Petch as *B. bassiana* (Bals.) Vuill.(15)

OBSERVATIONS ON THE INCIDENCE OF FUNGOUS PARASITES ON THE COCONUT LEAF MINER IN THE INFESTED AREAS

Contrary to the opinion or observations of previous investigators,(10, 13, 16) that when an insect pest has reached its maxi-

mum numbers or becomes excessively numerous a fungous epidemic generally ensues, did not hold true with the coconut leaf miner. As far as the author's observation is concerned, made during the months of March to October, 1930, and corroborated verbally by Messrs. F. Q. Otanes, S. S. Gonzales, P. Sison, entomologists, and other field personnel of the Bureau of Plant Industry, engaged in the leaf-miner eradication campaign in Laguna, Batangas, and Tayabas Provinces and other infested regions, there was no serious mycotic epidemic on the coconut leaf miner. The occurrence of a few isolated cases of inactive fungus pathogenes that the writer noticed in his trips to the coconut leaf-miner-infested localities, together with several specimens that came to the writer's attention through the courtesy of Mr. Gonzalo Merino, chief entomologist of the Bureau of Plant Industry and chief executive of the coconut leaf-miner eradication campaign, seems to indicate that the climatic and other environmental conditions then obtaining were unfavorable to their proper development. According to Petch,(16) the factors that govern the outbreak of fungous epidemics upon insects rest on a condition of the insects which renders them more liable to fungous attack. Charles(6) states that for their optimum growth and development they demand a high degree of moisture and temperature.

Judging from previous reports, therefore, together with the writer's personal experience, it would seem that the practical application of entomogenous fungi depends greatly on the circumstances obtaining in a particular place, such as the prevalence of the right weather and climatic conditions for the progress of the fungus, the relative numbers of the host insects, the gregarious habit of the insect, and withal the activity or virulence of the fungus. No less important are the methods and technic, such as the discovery of the most suitable media, use of the fungus in large doses, method of distribution and application, etc.

PARASITISM TESTS

ARTIFICIAL INFECTION OF THE COCONUT LEAF MINER UNDER GREENHOUSE CONDITIONS

Inspired by the belief that the locust fungus might be of some help in combating the coconut leaf miner, despite the apparent absence of natural outbreaks of fungous epidemics, the following experiments were undertaken. Its parasitism was therefore first proven in the greenhouse before attempting to use it in the open

or in the natural habitat of the insects. If the disease were to be introduced into the field it was absolutely essential that one should know first if it would prove pathogenic to the eggs, larvæ, pupæ, or adult, to the host plant, or to the insect parasites of the coconut leaf miner. Also, one should find out beforehand the most feasible method of disseminating the fungus. In other words the main objectives in these experiments were to find out the infective capacity of the fungus on the coconut leaf miner, and to devise a suitable technic and method of spread for its practical application.

Experiment 1. Dusting coconut leaf-miner beetles with spores.—The first greenhouse [2] attempt to infect healthy beetles of the coconut leaf miner,[3] fresh from the coco groves in Tanawan, Batangas Province, was made January 22, 1930, at 3 p. m. Eighty-two beetles were placed in large, sterilized glass jars covered with sterilized, copper-wire gauze and were then dusted with spores dislodged from a fresh culture, seeing to it that all the insects came in contact with them. Eighty-two other beetles were subjected to the same conditions, without the application of spores, and used as control. Each jar was provided with food by inserting equal numbers of fresh green portions of coco-leaf pinnæ, their fresh-cut ends being submerged in sterile water contained in a small beaker to keep them as fresh as possible; the pinnæ were replaced daily by fresh ones. In order to preclude outside infection, the leaves, before cutting in pieces, were wiped with cotton moistened with mercuric bichloride solution (1 : 1000) and then washed with sterile water. To prevent marauding insects, such as ants, from gaining access to the jars, these were set in shallow pans containing a solution of Doctor Bode's bacillol.

The next morning the fungus spores were observed adhering to the body and elytra, but were more noticeable on the tarsi. The inoculated insects were more or less sluggish and not as active as those in the control jars. They seemed to have lost their desire to eat, and very few were actually seen on the leaves. The majority of the insects were found at the bottom of the

[2] This greenhouse has a glass roof and metallic-cloth sides so that there is a free circulation of air. At the time of the experiment there was very little sunlight admitted because of the branches of a large rain tree, *Samanea saman* (Jacq.) Merr., shading it.

[3] Obtained through the courtesy of Mr. J. M. Mendoza, associate mycologist of the Bureau of Science.

jar in a weakened condition, and very few had the desire to escape through the wire-gauze cover. By comparison, the control insects were very active and the moment the wire gauze was removed they would crawl up quickly to the opening in an effort to fly away. Many were seen feeding on the leaves while some stayed at or near the mouth of the jars waiting for a chance to escape. The final results of the observations, showing the number of casualties in each jar, are given in Table 1.

TABLE 1.—*Infection of adult coconut leaf miners by dusting with spores.*

Treatment.	Started.	Beetles.	Total mortality.			Beetles showing the fungus distinctly at the end of experiment.	Infection.
			Jan. 23.	Jan. 24.	Jan. 25.		
							Per cent.
Dusting	Jan. 22, 1930	82	21	67	73	46	[a] 63.0
Check	----do----	82	1	7	22	0	0.0

[a] Based on the total mortality observed January 25, 1930.

Seventy-three of the inoculated insects died in three days, while many of those in the control jar remained alive for a much longer period and the daily mortalities were much fewer. Disregarding the number of insects that presumably died of drowning, as they were found in the beaker of water supporting the coco-palm leaflets, and the few that were accidentally crushed or pinned between the glass jar and the wire-gauze cover, and as they showed no outward signs of infection, making a total of thirty-seven in the control lot and nine in the dusted, twenty-two died in the uninfected lot and seventy-three in the inoculated lot in three days. Due to the greater activity of the insects in the control lot, more insects died of drowning and by accident. Of the seventy-three individuals that died in the inoculated lot forty-six, or 63 per cent, showed distinct, identical fungous growths, which is considered here the actual number or percentage of infection. There seems, however, little doubt that more than forty-six beetles actually succumbed to infection, but because of mild attacks the outward manifestations of the fungus were more or less arrested by unfavorable environmental factors. Reisolations proved positive. No fungus appeared on the beetles that died in the control jar. Some of the control beetles survived until February 24, 1930, and eggs were found on the leaves after January 27, 1930.

The beetles collected from each jar were incubated individually in separate sterilized test tubes plugged with cotton. In about three days fungous growths were visible with the naked eye, coming out of the different parts of the carcass. With the aid of a hand lens these could be seen earlier.

The infected carcasses lose much of their lustrous appearance and become dull and darker brown ocher, while the controls retain their luster and much of their natural color (Plate 1). The fungous growths were more conspicuous ventrally than dorsally, and in some instances they covered much of the ventral surface (Plate 2). Generally the fungus came out more densely from the axillæ and at the juncture of the legs with the body segments. It was also visible at the joints of the legs, neck, the intersection of the segments, and at the extremeties (Plate 2). Occasionally the tarsi and the antennæ were attacked, but the elytra were seldom affected. The action of the fungus on the coconut leaf miner is more or less the same as on the locust.

The development of the fungus on the carcass was observed to be variable; on some the growth was luxuriant, on some moderate or fair, while on others it was scanty. This seems to indicate that weather conditions have a direct bearing on the behavior and growth of the organism. The differences might be ascribed also to the variation in the health or resistance of the individual insects.

Experiment 2. Spraying adult coconut leaf miner with spores in suspension.—In another set of experiments infection was carried out by spraying the beetles[4] with a spore suspension from a fine De Vilbiss atomizer. The sprayed beetles, numbering eighty-two, were kept in large glass jars and supplied with plenty of fresh portions of coco-palm pinnæ, following exactly the same procedure and technic as in the preceding experiment, except that the beaker of water supporting the coco-palm leaflets was covered with sterile cotton at the top to minimize the deaths due to drowning. The control beetles, also eighty-two in number, were atomized with sterile distilled water and kept under conditions similar to the inoculated ones. The bodies from both lots collected after each observation were kept singly in separate sterile test tubes plugged with cotton. The data given in Table 2 show the results of observation.

[4] Furnished by Mr. Pedro Sison, of the Bureau of Plant Industry, and collected in Laguna Province.

TABLE 2.—*Infection of leaf-miner beetles by spraying with a spore suspension.*

Treatment.			Mortality. 1930.					Beetles showing the fungus distinctly at the end of experiment.	Infection.
Kind.	Started.	Beetles used.	Feb. 8.	Feb. 10.	Feb. 11.	Feb. 12.	Feb. 13.		
									Per cent.
Spraying	Feb. 7, 1930	82	4	31	60	70	71	52	[a] 73.2
Check	----do----	82	3	24	40	49	51	0	0.0

[a] Computed on the total mortality observed February 13, 1930.

From the above data it will be seen that the percentage of beetles visibly infected as a result of spraying is much higher than the percentage of infected beetles in the dusting experiment. Of a total of eighty-two beetles sprayed seventy-one were killed, but it took five days, of which fifty-two, or 73.2 per cent, showed a marked external growth of the inoculum on their carcasses. Eleven were either drowned or accidentally killed. Although most of the insects died, those that showed no trace of the fungus were considered uninfected or to have died a natural death. There is no concrete evidence to show that all those that died in the sprayed lot were killed by the fungus, and no attempt was made to dissect all the dead insects showing no fungus evidence, owing to their smallness and their tendency to break easily into fragments, or become dismembered, under slight pressure. Nevertheless, the few cases successfully dissected longitudinally showed fungous growths, which, when cultured, yielded a species of *Aspergillus*.

Fifty-one of the control insects died, but some lived for a longer time than those inoculated. No fungus was observed. Twenty-five of the beetles were either drowned or killed by accident and two escaped.

Owing to the close similarity of the growth of the fungus on the victims (Plate 3) only a few reisolations were made from the doubtful cases and these gave positive results. Spraying evidently proved more effective than dusting with spores, probably because the spores were provided with extra moisture, which facilitated germination and initial infection.

Experiment 3. Infection of healthy coconut leaf-miner beetles[5] *by contact with inoculated ones.*—In this experiment, which was conducted in duplicate, it was the aim to create a sort of epidemic or infect healthy living specimens by mixing with them a few inoculated ones. Ten active beetles were selected and smeared with a thick spore suspension of the fungus in a sugar solution and then released in a sterilized glass jar containing a certain number of disease-free beetles. The same procedure and aseptic precautions were followed as in the foregoing experiments, excepting the method of infection. All dead beetles from the four jars were removed and kept in separate sterile test tubes with cotton plugs for further examinations. The number of beetles used in these tests were not the same because of the extreme difficulty in counting large numbers, and in order to avoid unnecessary handling. The results are shown in Table 3.

TABLE 3.—*Showing the results of infecting healthy adult coconut leaf miners by introducing with them inoculated ones.*

Treatment.				Total mortality.				Beetles showing the fungus distinctly.	Infection.
Kind.	Started.	Beetles used.	Beetles inoculated.	Mar. 17.	Mar. 18.	Mar. 19.	Mar. 22.		
									Per cent.
Natural contact	Mar. 15, 1930	99	10	53	64	64	69	8	[a]11.6
Control	do	95	0	39	44	44	46	0	0.0
Natural contact	do	104	10	49	62	65	68	5	[a]7.3
Control	do	122	0	56	60	61	[b]62	0	0.0

[a] Based on total mortality observed March 22, 1930.
[b] Two beetles were attacked by a species of *Aspergillus*.

The few cases of successful infection that resulted from the foregoing experiment proved this method to have no appreciable effect. In the first set only eight beetles developed the disease while in the second set only five were infected, indicating that probably no contamination took place, and not even the ten beetles introduced with the fungus in each set of experiment were all killed. This seems to show that with this particular insect pest, the possibility of artificial transmission or spread of the fungus by natural contact is very meager, if not altogether lacking. As far as the writer's observation is concerned, the only possible chance of contact is when a healthy beetle copulates

[5] Obtained through the courtesy of Mr. F. Q. Otanes, entomologist of the Bureau of Plant Industry, around San Pablo, Laguna Province.

with an infected one, or when a relatively large number of the insects are confined in cramped quarters, so that the live beetles pass over the dead remains. Wind may blow spores from infected insects and, by chance, some of such spores may lodge on uninfected beetles. The same fungus was recovered in reisolations.

ARTIFICIAL INFECTION OF THE COCONUT LEAF MINER IN LARGE WIRE CAGES PLACED IN THE OPEN

The results of infection experiments conducted in the laboratory have often been criticized because the conditions under which the insects were kept could not be called normal, and the methods evolved could not be applied in practice on a large scale. With this in mind, experiments were undertaken outdoors where fresh and usually drier air prevails, in order to simulate nature as much as possible and at the same time approach, as far as is humanly possible, an efficient method.

The larval and pupal stages of the coconut leaf miner are passed in excavations, made by the larvæ in the parenchyma of the leaves between the upper and lower epidermis, so that they are more or less protected from the attack of nonmotile organisms. The use of fungous parasites, therefore, is somewhat at a disadvantage in this respect.

Large wire-screen (1/16-inch mesh) cages, made for the purpose were used. The cages were 1.5 meters high, 90 centimeters wide, and 90 centimeters deep. The wooden frame-work consisted of narrow strips in order to secure normal aëration as far as possible (Plate 5). The legs were set in shallow vessels containing a solution of Dr. Bode's bacillol to prevent ants and other insectivorous animals from gaining entrance. After sterilizing the cages, two healthy and vigorous coco-palm seedlings, about 2.5 meters high, free from leaf-miner attack and other blemishes and planted in sterilized soil in pots, with the leaves having been previously sterilized, were set on the wooden floor of each cage (Plate 5). All the cages were placed in the open but were so arranged that they received no sunlight after 1 o'clock in the afternoon. In order to assure the use of almost sterile beetles, trips were made to the leaf-miner regions to secure new broods as much as possible, and some were bred by the writer in a large wire cage, where the stock of beetles were released and fed before using. For every experiment therefore a fresh supply of beetles was used, and these were collected from the coco-palm groves not treated with insecticides. All outdoor

infections were done in the afternoon in order to maintain a certain amount of humidity about the experimental cages and to avoid rapid drying of the solution enveloping the microspores. Thus, the experiments were as nearly normal as possible.

Experiment 1.—In this infection experiment fifty healthy and active beetles [6] were selected for each of the two treatments and fifty for the control. Two methods of inoculation were employed: First, by dipping twenty beetles for a few seconds in a suspension of spores in syrup solution made by mixing copious spores in 1 cubic centimeter of 2:1 boiled syrup with 20 cubic centimeters of sterile distilled water, and then releasing them in cage 1 containing thirty healthy beetles supplied with abundant fresh food. Second, by spraying the lower surfaces of the sterilized leaves in cage 2 with a spore suspension until they were visibly wet before liberating inside fifty beetles, collected in a sterile test tube with the help of a curved, flexible forceps. The spores used for this experiment were obtained from a 42-day-old potato-glucose-agar culture. In the control cage, or cage 3, which was placed some distance away, the lower surfaces of the sterilized coco leaves were atomized with sterile water and twenty of the beetles were dipped in sterile water before releasing them with thirty others. The dead or dying beetles were collected individually in separate sterile test tubes provided with sterilized cotton plugs after each observation. The percentages of infection resulting from the two methods are presented in Table 4.

From Table 4 it will be seen that the chance of artificially propagating the fungus by natural contact is very meager, despite the proportion of beetle carriers to facilitate contacts. The result was not very encouraging as only two beetles were contaminated. This may be attributed partly to the fact that the insects do not wander en masse. It is believed, however, that if more beetles were used more would be proportionately infected. On the other hand, the spraying of the undersurfaces of the leaves proved more successful, causing 48 per cent infection. It was about a month before all the beetles in cages 1 and 2 died. As some of the beetles in the control cage lived for a longer time than in the treated cages, the experiment was concluded August 20, 1930, when three were still alive. In the

[6] Obtained through the kindness of Mr. S. S. Gonzales, assistant entomologist of the Bureau of Plant Industry, from Lipa, Batangas Province.

TABLE 4.—*Result of artificial infection of adult coconut leaf miner by dipping in spore suspension in syrup solution, or spraying the lower surfaces of the coco leaves with spores diluted with sterile water.*

Set.	Cage No.	Beetles used.	Treatment.	Days observed.	Mortality.		Beetles showing fungous growths.	Infection.	Remarks.
					Beetles died.	Inclusive dates.			
								P. ct.	
July 1, 1930	1	50	Dipping 20 beetles in spore suspension before mixing with the others.	32	50	July 2 to August 2, 1930.	22	44.0	Six beetles pasted by the fungus on the leaves.
	2	50	Spraying the leaves before allowing beetles to eat.	30	50	July 5 to 31, 1930.	24	48.0	Nine beetles pasted by the fungus on the leaves and three on wire. One accidentally killed.
	3	50	Control; 20 beetles dipped in, and leaves sprayed with, sterile water.	50	47	July 19 to August 20, 1930.	None.	0.0	Three beetles still living August 20, 1930.

treated cages some of the beetles that perished without losing their hold on the leaves were held fast to the leaves by a copious growth of fungus on their bodies (Plate 4), and three were found held to the wire-screen wall of cage 2 by the fungus that covered them, although the majority dropped to the floor. The hypogenous attack on the leaves was much in evidence by the longitudinal scars present, and eggs were laid, especially on the leaves in the control cage. The insects that died as a result of infection were not readily recognized until they displayed conspicuous mycelial outgrowths from their body sutures. A similar diseased condition invariably developed in the infected beetles, while none of the untreated ones showed any evidence of fungus. Reisolations agreed in all respects with the culture used as inoculum.

From the result of the second part of the experiment, there seems to be no doubt that the spores in the leaf tissues ingested by the beetles live within and penetrate the insect tissues through the chitin integument, developing profusely on the surface. A more liberal use of the fungus would probably have more

disastrous effects. Dufrénoy,(8) in his studies on the transmission of plant diseases through biological channels, found that some coleopterous insects harbor certain parasites in their alimentary canal, which multiply and remain during a part of their life cycle and cause infection when conditions become favorable. This author further states that in some instances the insect carriers become the victims of the fungi they carry, such as *Beauveria*.

Experiment 2.—Another method of infection has been tried in this experiment; that is, by spraying coconut leaf-miner beetles [7] while they were feeding on the leaves with spores suspended in sterile water, using a fine atomizer. The experiment was begun October 11, 1930, using spores obtained from a 30-day-old culture on leaf-miner decoction agar.[8] The beetles in the control cage were atomized with sterile water. All precautions used in the preceding experiment were observed carefully. The results are recorded in Table 5.

TABLE 5.—*Artificial infection of leaf-miner beetles by spraying them on the leaves they ate with a spore suspension.*

Cage No.	Date.	Beetles used.	Treatment.	Observations.			Infection.	Remarks.
				Dates.	Total mortality.	Beetles showing fungous growths.		
							P. ct.	
1	Oct. 11, 1930	50	Beetles sprayed on the leaves they eat with spore suspension.	Oct. 13 to 21, 1930.	43	29	[a]67.4	Four pasted by the fungus on the leaves.
2	Oct. 11, 1930	50	Control; sprayed with sterile water.	Oct. 18 to Nov. 25, 1930.	40	0	0	None.

[a] Based on total mortality.

Of the fifty beetles sprayed forty-three died. Twenty-nine of these showed the presence of the inoculum used, and four of them were found pasted by the fungus on the undersurfaces of the

[7] From a new brood of adult coconut leaf miner collected at Lumut, Calauan, Laguna Province.

[8] This medium is a mixture of 100 grams pounded leaf-miner beetles (oven-dried), 15 grams agar-agar, 20 grams dextrose, and 1,000 cubic centimeters distilled water.

leaves. Taking into account only those that showed the presence of the fungus, the rate of infection by this method was 67.4 per cent, which, by comparison, is considerably higher than in the preceding experiments, conducted also under similar conditions, and the beetles succumbed much quicker. At the time this experiment was in progress, the Rev. Dr. Miguel Selga, director of the Weather Bureau, Manila, reported the prevalence of a prolonged warm, humid atmosphere,[9] which probably accounts, in part, for the high rate of mortality, which is also accentuated by the use of cultures of suitable virulence, and through exposure to both internal and external infection.

When the experiment was closed seven beetles were still living in the sprayed lot and ten in the unsprayed. None of the control beetles that died showed any trace of fungous attack. The action on the beetles is similar to that on its usual host. The same fungus was recovered in reisolations.

The above experiments show evident results and will lead to more extensive experimentation, but results are far from being invariably successful because success is contingent upon certain ecological factors beyond human control.

EXPERIMENTS ON THE EFFECT OF THE FUNGUS ON THE PLANT HOST

In view of the fact that *Sporotrichum globuliferum* Speg.(9) and other species have been observed to infect plants, tests were conducted with a view to finding whether or not the fungus would also affect the coco palms. July 15, 1930, two young, healthy coco seedlings, about 2.5 meters high, were sprayed by means of an atomizer, on the foliage and at the base of the youngest leaf with a large dose of spores diluted in sterile water, to the extent of drenching the growing point. The same process was repeated July 22, 1930, or just a week later. Another plant used as control was sprayed with sterile water.

From July 29 to October 18, 1930, when the experiment was closed, observations made at weekly intervals revealed that the fungus had no ill effects on the coco seedlings. Spores sprayed on the leaves and on the growing point apparently produced no disease symptoms. It proves that if the fungus is used extensively to control the leaf miner, there should be no fear of the fungus damaging the coco plants except perhaps through mechanical injuries, or when the fungus is transported through wounds

[9] Absence of typhoons now is cause of prevailing heat wave. The Tribune Year 6, No. 140 (1930) 12, Manila, Philippines.

TABLE 6.—*Observations on the effect of spores of Beauveria globulifera (Speg.) Pic. sprayed on coco-palm seedlings; sprayed July 15 and 22, 1930.*

Observations.[a]		
Date.	Seedling 1.	Seedling 2.
1930		
July 29	No effect	No effect.
August 5	do	Do.
August 12	do	Do.
August 19	do	Do.
August 26	do	Do.
September 2	do	Do.
September 9	do	Do.
September 16	do	Do.
September 23	do	Do.
September 30	do	Do.
October 7	do	Do.
October 14	do	Do.
October 18	do	Do.

[a] The control seedling remained healthy also.

or tunnels made by other insects, such as the red coconut weevil, *Rhynchophorus ferrugineus* Fabr., which has been declared by Petch(17) susceptible to the attack of either *Beauveria globulifera* or *B. bassiana* in specimens he obtained from Ceylon.

TESTS FOR LONGEVITY OF SPORES SPRAYED ON THE LOWER SURFACES OF COCO LEAVES

August 11, 1930, spores from a 20-day-old culture of *Beauveria globulifera* (Speg.) Pic. were mixed with 2 per cent syrup solution and 2 per cent gum arabic solution and sprayed separately on the lower surfaces of the leaves of two coco-palm seedlings. Before spraying, the leaves were wiped with cotton moistened with 1 : 1000 mercuric chloride and later washed thoroughly with cotton drenched with sterile water. At certain intervals spores adhering to the lower surfaces of the leaves were tested for viability, and at the close of the experiment the sprayed leaves were examined for symptoms that might have been caused by the fungus. The results are recorded in Table 7.

Table 7 shows that the viability lasted sixty-nine days on the leaves sprayed with spores diluted with 2 per cent syrup solution and a much shorter time on the leaves sprayed with spores mixed with 2 per cent gum arabic solution. Gum arabic and syrup were used not so much with the view to prolonging the life of

TABLE 7.—*Tests for the longevity of spores of Beauveria globulifera (Speg.) Pic. in suspension, sprayed on the lower surfaces of the coco-palm leaves.*

Plant No.	Vehicle used in spraying spores.	Culture.	
		Age.	Used.
1	Syrup (2 : 1) diluted with sterile water 2 per cent.	Twenty-day old on potato glucose agar+1 F. S.	Aug. 11, 1930.
2	Gum arabic, 2 per cent in sterile water.	----do----------	Do.

Plant No.	Date tested and observation.				Remarks.
	Aug. 19, 1930.	Sept. 11, 1930.	Sept. 25, 1930.	Oct. 18, 1930.	
1	Viable--------	Viable--------	Viable--------	Viable--------	No disease developed.
2	----do--------	----do--------	None----------	None----------	Do.

the organism but mainly to serve as adhesives. The syrup solution in this experiment apparently served the dual purpose intended and gave the better result. A further test would probably show that the spores will remain viable for a much longer period, especially if they are applied more abundantly and spraying is done more liberally, so that a larger quantity of the spores stick to the leaves and facilitate detection. As the spores constitute the chief means of propagation, the significance of the test cannot be too strongly emphasized as on it will depend to a large measure the success of the agricultural application of fungous diseases against injurious insects destructive to, or feeding on, the leaves, under normal conditions or in their native habitat. This test again proved the harmlessness of the fungus to the plant host.

EFFECT OF THE FUNGUS ON THE HYMENOPTEROUS PARASITES OF THE COCONUT LEAF MINER

Of the natural enemies of the coconut leaf miner that played a prominent rôle there are six species of hymenopterous parasites, commonly called larval parasites, preying on the larvæ and pupæ.[10] To determine the susceptibility of these tiny insects to *Beauveria globulifera*, three experiments were conducted in the laboratory.

[10] Information obtained from Mr. Pedro Sison, assistant entomologist of the Bureau of Plant Industry, March 13, 1930.

EXPERIMENT I

March 13, 1930, a few newly emerged larval parasites were obtained from Mr. Pedro Sison, assistant entomologist of the Bureau of Plant Industry at San Pablo, Laguna Province. Six of these tiny insects were confined in a sterilized 1-inch-diameter test tube plugged with cotton and were supplied with sugar-solution food placed on a piece of paper in small droplets. Four others were placed in another test tube of the same size and were given the same treatment. These tubes were placed horizontally on a table near a window. March 19, 1930, in the afternoon, six insects in one of the tubes were transferred into a sterile test tube, the inner wall of which was literally covered with a thin layer of spores of *Beauveria globulifera*. They were allowed to roam about for a few minutes until spores could be seen clinging to them, with the aid of a hand lens, when they were transferred again to the tube from whence they came. The other four insects in the other tube were used as controls. Great care was taken that none of the insects received injury of any kind in handling. The setting of the experiment was done expeditiously to avoid contamination from the air. As the insects are attracted by the light, their transfer from one tube to another was facilitated by pressing the mouths of the tubes together and placing the bottom of the tube to which the insects were to be transferred towards the light. In this way the insects crawled to the tube receiving more light and were thus transferred easily from one tube to another. Dead insects were collected with a sterile platinum loop into separate sterile test tubes at each observation.

TABLE 8.—*Inoculation of hymenopterous parasites of the coconut leaf miner.*

Four insects untreated and used as check.		Six insects allowed to walk on spores.		
Date observed.	Number infected.	Date observed.	Number infected.	Reisolations.
Mar. 20, 1930	All living	Mar. 20, 1930	One died, 8 a. m. One died, 9 a. m.	*Aspergillus* sp.
Mar. 21, 1930	do	Mar. 21, 1930	Two died, 1.30 p. m.; fungus growth visible.	One positive; 1 *Aspergillus* sp.
Mar. 22, 1930	do			
Mar. 24, 1930	do	Mar. 24, 1930	Two died	*Aspergillus* sp.
Mar. 26, 1930	do			
Mar. 28, 1930	do			
Mar. 31, 1930	do			
Apr. 2, 1930	do			
Apr. 8, 1930	do			
Apr. 11, 1930	do			

As given in Table 8, the parasites that were artificially infected died in one to five days after the date of inoculation, while those uninfected lived for twenty-three days, or until April 11, 1930, when observations terminated. Reisolations were made from the inoculated insects of which only one showed the inoculum used, while the rest yielded a species of *Aspergillus*. The results seem to show that the organism is not an active pathogene of the leaf-miner parasites. The failure of the fungus successfully to infect more of the insects may be attributed to the habit of these tiny insects. Like the house fly they are very active and are capable of cleaning themselves by brushing off with their legs water droplets, spores, or any object clinging to their bodies. Being very small the few spores which adhered to them might have fallen of their own weight or through their jerky movements, especially when desiring to fly.

EXPERIMENT II

June 28, 1930, at 11.30 a. m., thirteen leaf-miner larval parasites obtained from Mr. S. S. Gonzales, at Lipa, Batangas Province, the previous day were transferred into a sterile 1-inch test tube containing spores of the fungus, and were allowed to roam around for about two minutes before transferring them into another 1-inch sterile test tube. Thirteen other larval parasites were placed in another large sterile test tube and used as controls. Diluted honey was supplied as food for the insects by putting small droplets on pieces of sterilized coco-palm leaflets.

TABLE 9.—*Artificial infection of larval parasites of the coconut leaf miner.*

Treatment.	Date set.	Insects in each treatment.	Mortality. 1930.			
			June 30.	July 2.	July 11.	July 14.
Inoculated	June 28, 1930	13	[a] 4	9		
Control	do	13	None.	12	None.	1

Treatment.	Date set.	Reisolations. 1930.	
		Inoculated.	Control.
Inoculated	June 28, 1930	July 11; 2 doubtful; 7 *Aspergillus* sp.	July 11; 5 *Aspergillus* sp.
Control	do	July 16; 4 *Aspergillus* sp.	July 16: 1 *Aspergillus* sp.

[a] One dying.

As shown in Table 9, four of the inoculated larval parasites died two days after inoculation and one of which was in mori-

bund condition. In two more days all the remaining insects in the inoculated tube perished, while twelve died in the control tube. The lone parasite remaining in the control tube lived until July 14, or sixteen days, from the time the experiment was started. When the reisolations were made from those showing the presence of fungus, two of the inoculated insects showed a white fungus associated with a species of *Aspergillus* but they were not very typical of *Beauveria globulifera*. The other insects gave negative results, while six of the control insects yielded a species of *Aspergillus*. The contaminating organism that developed on both the inoculated and control insects was probably present on them before the inoculations were made because the original locality from which the insects came was a very humid place.

EXPERIMENT III

Following the same treatment and method of inoculation a third trial was made. July 31, 1930, abundant spores obtained from a fresh culture of the fungus were spread on the inner surface of a 1-inch sterile test tube and into it were placed newly emerged parasites obtained from Mr. C. Buligan, of the Bureau of Plant Industry at Magdalena, Laguna Province, the previous day. Twenty-seven parasites were allowed to roam about for four minutes before allowing them to return to their former confinement—another large sterilized test tube. Diluted honey in fine droplets placed on pieces of sterilized coco-palm pinnæ was supplied as food. Thirty uninoculated parasites were released in the same-sized test tube and given the same food. The results are shown in Table 10.

It will be seen from the results tabulated that the inoculated insects died much sooner, while at the close of the experiment August 9, 1930, eighteen of the untreated insects were still alive and two remained healthy until September 7, 1930. Eleven insects showing fungous growth in the inoculated lot and two of the controls yielded a species of *Aspergillus* and were, therefore, negative. Just how these insects picked up the contaminating organism cannot be explained.

The three preceding experiments tend to show that the fungus is not an aggressive pathogene of the coconut leaf-miner parasites, although the tests were not so extensive as to warrant definite conclusions. There is little likelihood, however, that these so-called larval parasites would fall easy prey to the fungus because they are hidden with the larvæ and pupæ they attack

TABLE 10.—*Artificial inoculation of larval parasites of the coconut leaf miner.*

Treatment.	Insects used.	Date observed.	Mortality in inoculation.	Mortality in control.
		1930		
Inoculated	27	Aug. 2	Eleven died: 5 showed fungous growth; 2 died in honey.	None.
Check	30	Aug. 4	Thirteen died: 6 showed fungous growth; 1 died in honey.	Two died; no fungous evidence.
		Aug. 5		Three died; no fungous evidence.
		Aug. 6		One died in honey.
		Aug. 7		Three died in honey.
		Aug. 8		None.
		Aug. 9		Two died; 1 drowned, apparently.

Reisolations.	
Inoculated.	Control.
Eleven insects yielded mold; no fungus developed in the rest.	Two insects developed mold while the others none.

within the leaf tissues. In other words, these parasites are protected not only by the thick epiderma of the coco leaves but also by the coating of their hosts where they pass the greater part of their life. As these parasites become full grown, they pass out of the leaves through tiny punctures made in the epidermis and probably live on other hosts during this intervening period until they are able to lay eggs, or die without perpetuating their species. Many of them probably succumb to adversities of weather, while numerous others are perhaps destroyed by their predators and hyperparasites.

SUMMARY AND CONCLUSIONS

This paper reports primarily the results obtained from experimental attempts to infect artificially the adult coconut leaf miner, *Promecotheca cumingi* Baly, with a fungus which has proved identical with *Beauveria globulifera* (Speg.) Pic.

The opinion or observation of previous investigators that when an insect pest has reached its maximum numbers or becomes excessively numerous a fungous epidemic generally ensues, did not hold true with the coconut leaf miner. However, a few

isolated cases of inactive fungous pathogenes have been encountered. Their development and spread are greatly dependent upon weather and climatic conditions.

Several methods were employed with varying degrees of success in artificially inducing the disease on the coconut leaf miner; namely, by dusting the beetles with spores; by spraying them with spores in water suspension; by applying spores on some individuals and releasing them with the healthy ones (or by natural contact); by spraying the lower surfaces of the coco leaves they eat with a spore suspension; and by spraying the beetles with a suspension of spores while they are feeding on the leaves. Under natural conditions the last method proved to be the most effective, causing a high percentage of fatality, because of exposure of the insects to both external and internal infection. Spraying is also more feasible because the beetles do not usually fly in the daytime even if disturbed, except at dusk and at dawn.

The infectious nature of the fungus has been demonstrated by experiments conducted under properly controlled conditions. In tests conducted in large wire cages in the open, or under conditions approaching to some extent those found in nature, the organism proved a pathogene of considerable importance to healthy beetles of the coconut leaf miner, causing 44 to 67.4 per cent mortality. Under greenhouse conditions, positive results were also obtained, the mortality ranging from 7.3 to 73.2 per cent, depending on the method of inoculation or propagation employed. The course of the disease was the same as on its natural host.

The experiments carried out in the open were not extensive enough and further tests should be made in the coco-palm regions to determine more definitely the efficacy of this fungus before recommendations can be made.

That this parasitic locust fungus can be artificially communicated to healthy beetles of the coconut leaf miner evidently proves the pleophagous nature of the organism.

The viability of the spores of the fungus sprayed on the coco-palm leaves was found by tests to last two months and nine days, but further tests would probably reveal that the spores will remain viable for a much longer period, especially if spores are applied more abundantly to facilitate detection. The value of the test lies in the fact that the longer the spores remain viable the greater the chance for the insects to be infected.

Although *Sporotrichum globuliferum* and other species of *Sporotrichum* have been reported to infect many kinds of plants, the healthy coco palms seemed apparently immune to it.

On the whole, all attempts to infect the hymenopterous or so-called larval parasites have ended in failure, indicating that this fungus is probably not an aggressive pathogene of these tiny insects. There seems to be little fear of these beneficial insects falling ready victims to the fungus as they spend the greater part of their life cycle within the coco leaves and are thus protected, not only by their thick, more or less glossy or waxy epiderma, but also by the coatings of the larvæ and pupæ in which they hibernate. It is not definitely known where the parasites spend the rest of their mature life. Being short-lived animals it is presumed that they are either destroyed by their predators and hyperparasites or that they die a natural death after perpetuating their species.

LITERATURE CITED

1. Aldaba, V. C. The coconut leaf-miner infestation of Laguna, Batangas, and Tayabas. Philip. Journ. Agr. 1 (1930) 145–164.
2. Anon. Report of the prevalence of some pests and diseases in the West Indies during 1919: Diseases of economic plants. West Indian Bull. 19 (1921) 31–37.
3. Arnaud, Madeleine. Recherches préliminaires sur les champignons entomophytes. Ann. des Épiphyties 13 (January–February, 1927) 1–30, 2 figs., 2 diags., 13 graphs.
4. Billings, F. H., and P. A. Glenn. Results of the artificial use of the white-fungus disease in Kansas. U. S. Dept. Agr. Bur. Ent. Bull. 107 (1911).
5. Celino, M. S. Note: A fungous disease of the coconut leaf miner (Promecotheca cumingii Baly). Philip. Agr. 19 (September, 1930) 253.
6. Charles, V. K. Entomogenous fungi attack and destroy many harmful insects. U. S. Dept. Agr. Yearbook (1931) 211–214, fig. 49.
7. Dozier, H. L. Delaware Agr. Exp. Sta. Bull. 152 (Ann. Rept. 1926–1927). Dept. Ent. (November, 1927) 19–25.
8. Dufrénoy, J. La transmission des maladies des plantes par voie biologique. Rev. gén. des Sci. 32 (1921) 389. *Cited in* Rev. App. Myc. 1 (1922) 116–117.
9. Headlee, T. J., and J. W. McColloch. The chinch bug. Kansas Agr. Exp. Sta. Bull. 191 (1913) 287–353.
10. Howard, L. O. Ensayos sobre las enfermedades fungosas de las langostas. (Traducción por Sixto S. Sandejas de la edición Ingles.) Bol. del Agr. No. 6 2a. ed. (1904).
11. Le Moult, L. Le Hanneton et son parasite. Compt. rend. Acad. d'Agr. de France 8 (1922) 596–601. *Cited in* Rev. App. Myc. 1 (1922) 355.

12. LE MOULT, L. La destruction des insectes nuisibles par les parasites vegetaux. Rev. de Bot. Appliquée 3 (1923) 84–102. *Cited in* Rev. App. Myc. 2 (1923) 412–413.
13. MARTIN, H. The scientific principles of plant protection. Edward Arnold & Co., London (1928) xii + 316 pp.
14. NOWELL, W. Diseases of crop plants in the Lesser Antilles. Imperial Dept. Agr. The West India Committee. London (1923?) xix + 383 pp.
15. OCFEMIA, G. O. Notes on some economic plant diseases new in the Philippine Islands: II. (Fungous diseases of important insects.) Philip. Agr. 19 (1931) 581–589.
16. PETCH, T. Entomogenous fungi and their use in controlling insect pests. Ceylon Dept. Agr. Bull. 71 (1925) 40 pp., 2 pls. *Cited in* Rev. App. Myc. 4 (1925) 348.
17. PETCH, T. Studies in entomogenous fungi, VII. Notes on Beauveria. Trans. Brit. Myc. Soc. 10 (1926) 244–271, 1 fig.
18. REYES, G. M. An unreported fungous disease of the Philippine migratory locust. Philip. Journ. Sci. 49 (1932) 407–418, 5 pls.
19. VARGAS, JORGE B. The campaign against the coconut leaf miner. Philip. Journ. Agr. 2 (1931) 189–207.

ILLUSTRATIONS

PLATE 1

Coconut leaf miner beetles, *Promecotheca cumingi* Baly, infected by dusting with spores dislodged from a fresh culture of *Beauveria globulifera* (Speg.) Pic. (The presence of the fungus in the mass at the top can be seen clearer with the aid of a magnifier.) Compare the dull appearance of the infected beetles with the shiny and darker, control beetles at the bottom. Photographed six days after date of infection. Approximately × 1.6.

PLATE 2

The eight coconut leaf-miner beetles to the left show the characteristic growth of the inoculum, indistinguishable from one another, distributed irregularly in patches over the head and ventral side, in comparison with the uninoculated ones to the right. About 2.5 times natural size.

PLATE 3

Three adult coconut leaf-miner beetles, greatly enlarged, to show conspicuously the fungus produced by spraying with spores in suspension. Note the luxuriant, cottony-white growth of fine filaments enveloping the insects and forming a more or less furry coating, especially over the body sutures.

PLATE 4

Coconut leaf-miner beetles stuck fast by the fungous growth to the lower surfaces of coco-palm pinnæ as a result of artificial infection, dying in their natural position. The beetles on portions of two leaflets to the right were a result of dipping the beetles in spore dilution while the other beetles on the two other leaflets to the left were a result of spraying the undersurfaces with a spore suspension before allowing the beetles to eat. The narrow longitudinal scars on the leaves show the hypogenous attack. Natural size.

PLATE 5

A sample of the cages used for outdoor infection of the coconut leaf-miner beetle, having narrow strips of wooden frame-work, a wire top and sides and a wooden floor, measuring 1.5 meters high, 0.9 meter wide, and 0.9 meter deep. The legs stand in shallow dishes containing lysol solution.

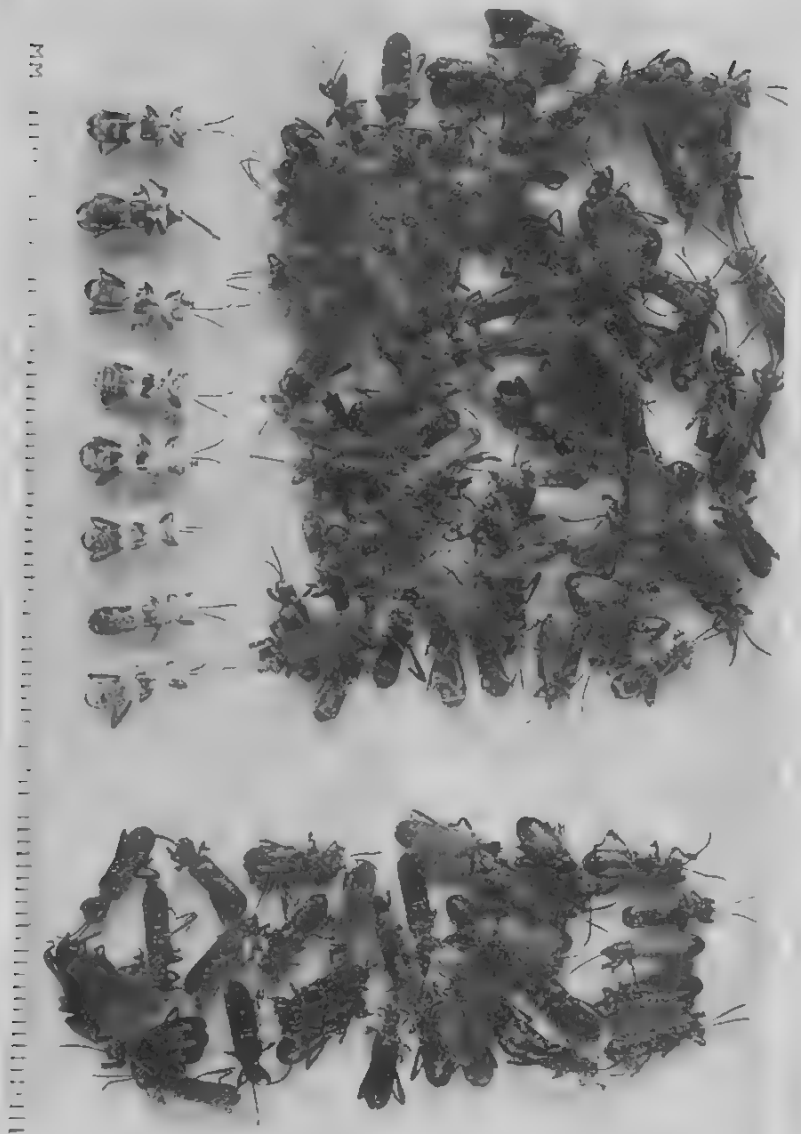


PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

PLATE 5.

THE PHILIPPINE SPECIES OF PARASTERINA

By José Miguel Mendoza

Of the Division of Botany, Bureau of Science, Manila

FIFTEEN PLATES

In this paper sixteen species of Philippine *Parasterina* have been studied, two of which are new. A key for the species has been prepared, as well as a short key to include the genera closely related to *Parasterina*. All descriptions and illustrations have been prepared from specimens available in the herbaria of the Bureau of Science, Manila, and the College of Agriculture, University of the Philippines, Los Baños, Laguna.

INTRODUCTION

The family Microthyriaceæ, to which the old genus *Asterina* and the new genus *Parasterina* belong, constitutes a very large group of fungi generally found in great abundance in the Tropics. They are mycologically interesting on account of their color shades, which vary from a beautiful transparent green to amber or dark brown, and because of the different shapes of the numerous hyphopodia that are present in many of the genera.

HISTORY

The genus *Asterina*, from which *Parasterina* was separated, is an old one founded by Léveillé.[1] In earlier work this was interpreted to include the paraphysate species now placed in *Parasterina*. These became so numerous, however, that the new genus was established to include them.

There have been diverse opinions as to what species should be taken as the generic type of *Asterina*. Theissen[2] first proposed that *A. azaraе* be adopted since Léveillé's type, *A. melastomatis*, is missing, and since the specimens named by Léveillé and deposited in different herbaria are not in accord. Subse-

[1] Ann. Sci. Nat. 3 (1845) 59.

[2] Fragmenta brasilica V nebst Besprechung einiger Microthyriaceen, Ann. Mycol. 10 (1912) 164.

quently, Theissen [3] advocated that the Parisian collection of *A. melastomatis* be regarded as the type, but for certain plausible reasons he finally decided that it would be better to return to his original proposal, and take as the type *A. azarae*, a species that has no paraphyses. H. and P. Sydow [4] supported Theissen's idea and added further that if the paraphysate *A. melastomatis* were taken as the type of the genus *Asterina* only a very small number of species could be included under it, while many, if not all, of those without paraphyses would be included under the genus *Dimerosporium*, where *D. abjectum* Fuck. (= *Asterina veronicae*) is the representative type. This would obviously be faulty since over one hundred currently accepted species of *Asterina* would come under *Dimerosporium* as now understood, this being an entirely different genus established long before. It was, therefore, decided to divide all species of *Asterina* into two groups; those without paraphyses with *A. azarae* (*Dimerosporium abjectum* Fuck.) as the type are to be retained under the genus *Asterina* and those with paraphyses are to be assigned to a new genus *Parasterina* of which *Parasterina melastomatis* (Lev.) Theiss. (= *Asterina melastomatis*) is the type.

In order to distinguish the genera that are closely related to *Parasterina*, a key patterned after that of Theissen [5] is presented.

Key to the genera.

1. Mycelium present, hyphopodia or knotted mycelial hyphæ present.
   2. Paraphyses present ..... *Parasterina.*
   2. Paraphyses absent ..... *Asterina.*
1. Mycelium present; hyphopodia absent.
   2. Spores brown ..... *Asterinella.*
   2. Spores colorless ..... *Calothyrium.*
1. Mycelium wanting.
   2. Spores brown ..... *Seynesia.*
   2. Spores colorless ..... *Microthyrium.*

Genus PARASTERINA Theissen and Sydow

Parasterina THEISSEN and SYDOW, Ann. Mycol. 15 (1917) 420.

Mycelium present, superficial, with hyphopodia or knotted cells; mycelial conidia one-celled or absent. Fruiting bodies

[3] Cfr. die Gatung Asterina, Abh. der K. K. Zooligisch Botanischen Gessell Shaft Band 7 (1912–1913) 213.

[4] Ann. Mycol. 15 (1917) 245–246.

[5] Le genre Asterinella, Brotéria: Série Botanica 10 (1912) 104–124.

roundish, flat, or hemispherical, radial, with central opening, radially splitting in the process of development, the surface very often sloughing off to a considerable degree. Hypothecium flat or somewhat concave, colorless. Basal membrane present or absent. Hymenium simple, with many asci. Asci ovoid to globose, thick walled, 8-spored. Paraphyses always present, often thickened at the top, slimy and agglutinate, forming a distinct epithecium. Spores brown, 2-celled.

Key to the species of Parasterina Theissen and Sydow.

1. Spores either spiny, warted, or with a roughened gelatinous surface.
  2. Spores spiny.
    3. Spines slender; hyphopodia 1-celled, abundant, distinctly lobed. 15. *P. spinosa.*
    3. Spines coarse; hyphopodia 1-celled, few, never lobed. 16. *P. tayabensis.*
  2. Spores warted.
    3. Hyphopodia 1-celled, rare, apex rounded or slightly lobed. 4. *P. dilleniae.*
    3. Hyphopodia 2-celled, numerous, apex distinctly lobed. 8. *P. jasminicola.*
  2. Spores with a roughened gelatinous surface, 2- to 4-lobed. 3. *P. cipadessae.*
1. Spores smooth.
  2. Spores subacute at both ends, cylindrical..... 2. *P. canthi.*
  2. Spores rounded at both ends, either elliptical or oblong.
    3. Hyphopodia rounded at the apex to very slightly lobed.
      4. Perithecia often in clusters, cylindrical, occasionally rounded; spores 15 to 17 μ long, 8 to 9 μ wide..... 9. *P. litseae.*
      4. Perithecia not in clusters, rounded; spores 18 to 25 μ long, 10 to 12 μ wide ..... 1. *P. astroniae.*
    3. Hyphopodia distinctly lobed but not flabellilobed.
      4. Perithecia usually long, cylindrical, occasionally rounded. 7. *P. homalomenae.*
      4. Perithecia rounded.
        5. Spores 19 to 21 μ long, 9 to 11 μ wide..... 10. *P. momordicae.*
        5. Spores 14 to 16 μ long, 6 to 8 μ wide..... 14. *P. samarensis.*
    3. Hyphopodia flabellilobed..... 11. *P. nycticaliae.*
    3. Hyphopodia never lobed, rounded at the apex.
      4. Perithecia rounded to cylindrical.
        5. Spores banded ..... 12. *P. pemphidioides.*
        5. Spores bandless ..... 13. *P. ramosii.*
      4. Perithecia rounded, never cylindrical.
        5. Hyphopodia long, cylindrical, 13 to 16 μ long; asci ovoid; spores 21 to 25 μ long, 11 to 12 μ in diameter..... 6. *P. fagarae.*
        5. Hyphopodia short, cylindrical, 10 to 12 μ long; asci oblong to ovoid; spores about 20 μ long, 5 μ in diameter. 5. *P. eugeniae.*

1. PARASTERINA ASTRONIAE (Yates) comb. nov. Plate 1, figs. 1 to 5.

Asterina astroniae YATES in Philip. Journ. Sci. Bot. 12 (1917) 370.

Fungous colony forming suborbicular to round, marginate spots, 4 to 8 millimeters in diameter, oftentimes confluent. Mycelium black, composed of a few, irregularly branching, septate hyphæ 3 to 4 μ wide; branches anastomosing. Hyphopodia unicellular, few, irregularly distributed, ovoid, oblong, generally slightly lobate, 5 to 6 μ long, 4 to 6 μ in diameter. Perithecia numerous, suborbicular to round, 160 to 200 μ in diameter; walls splitting, composed of dark brown hyphæ, 2 to 4 μ wide; periphery slightly fringed.

Asci subglobose to ovoid, 45 to 55 μ long, 28 to 34 μ in diameter. Spores heaped together, oblong, rounded at both ends, constricted at the single septum, dark brown, frequently with the cell contents contracted, 18 to 25 μ long, 10 to 12 μ in diameter.

SAMAR, Catubig River, *Bur. Sci. 24269 M. Ramos* (type in Bur. of Sci. herb.), March 21, 1916, on *Astronia* sp.

2. PARASTERINA CANTHI (Yates) comb. nov. Plate 2, figs. 1 to 5.

Asterina canthi YATES in Philip. Journ. Sci. Bot. 13 (1918) 372.

Forming epiphyllous, black, irregular, crestlike spots, 5 to 6 millimeters in diameter, sometimes covering the whole surface of the leaf. Mycelium abundant, attached to the surface of the leaf, composed of dark brown hyphæ, 5 to 8 μ wide; branches anastomosing, opposite to alternate. Hyphopodia very numerous, opposite, unicellular, oblong to cylindrical, rounded at the apex, about 5 μ long, 4.5 μ wide. Perithecia round, black, opaque; periphery fringed, 90 to 120 μ in diameter; texture subparenchymatous, the wall splitting irregularly.

Asci usually cylindrical, occasionally oblong, 30 to 40 μ long, 8 to 10 μ in diameter. Spores cylindrical, constricted at the single septum, dark brown, subacute at both ends, 10 to 12 μ long, 3 to 3.5 μ in diameter.

LUZON, Ilocos Norte Province, Burgos, *Bur. Sci. 27826 M. Ramos* (type in Bur. of Sci. herb.), March 2, 1917, on *Canthium* sp.

3. PARASTERINA CIPADESSAE (Yates) comb. nov. Plate 3, figs. 1 to 5.

Asterina cipadessae YATES in Philip. Journ. Sci. Bot. 12 (1917) 371.

Epiphyllous, forming black spots, at first 2 to 4 millimeters, but soon running together and more or less covering the surface of the leaf. Mycelium composed of branched, anastomosing hyphæ, 5 to 7 μ wide. Hyphopodia numerous, 1-celled, for

the most part opposite, sometimes alternate, or irregular, 2- to 4-lobed, 7 to 10 μ long. Perithecia numerous, 150 to 200 μ in diameter, dark brown, subopaque, usually globose, sometimes elliptical, walls splitting irregularly, composed of radiating, septate hyphæ, 3 to 4 μ wide, slightly fringed.

Asci ovoid, 35 to 45 μ long, 22 to 27 μ in diameter. Spores oblong, rounded at both ends, 1-septate, constricted at the middle, dark brown, rough, gelatinous, 28 to 32 μ long, 10 to 12 μ in diameter.

LUZON, Kalinga Subprovince, *Bur. Sci. 25307 H. S. Yates* (type in Bur. Sci. herb.), March 30, 1916, on leaves of *Cipadessa baccifera.*

Yates mentions in his description that the spores of this fungus are papillate. In the many examinations made on the type specimen no such papillate spores were seen; those found have a rough, gelatinous coating.

4. PARASTERINA DILLENIAE (Sydow) comb. nov. Plate 4, figs. 1 to 6.

Asterina dilleniae SYDOW in Philip. Journ. Sci. Bot. 9 (1914) 181.

Spots circular, 2 to 5 millimeters wide, dark gray, forming on the upper surface of the leaf. Mycelium radiating, composed of dark, chestnut brown, regularly spaced hyphæ, 7 to 9 μ long; branches with long beaks. Hyphopodia rare, alternate to one sided, 1-celled, globose, truncate to short cylindrical, chestnut brown, rounded at the end or somewhat lobed or angular, 10 to 15 μ long, 9 to 11 μ wide. Perithecia rare, flat, inverted, round, 140 to 200 μ in diameter; walls splitting, slightly black, opaque, composed of hyphæ, 4 to 5 μ wide.

Asci globose to ovoid, 40 to 50 μ long, 35 to 45 μ in diameter. Spores conglobate, oblong, broadly rounded at both ends, 1-septate, constricted at the middle, dark brown, warted, 20 to 25 μ long, 10 to 12 μ in diameter.

PALAWAN, Taytay, *Bur. Sci. 8774 E. D. Merrill* (cotype in Bur. of Sci. herb.), April 10, 1913.

5. PARASTERINA EUGENIAE (Yates) comb. nov. Plate 5, figs. 1 to 5.

Asterina eugeniae YATES in Philip. Journ. Sci. Bot. 12 (1912) 371.

Forming round to irregular, often marginate, spots up to 10 millimeters wide on the lower surface of the leaf, although sometimes on the upper surface. Mycelium effused, composed of a few, loose, dark brown, septate hyphæ, 4 to 5 μ wide; branches anastomosing. Hyphopodia few, alternate to irregular, cylindrical, rounded at the apex, 10 to 12 μ long, 5 to 7 μ wide.

Perithecia numerous, black, opaque, 225 to 274 μ diameter, perforated at the center; walls composed of radiating hyphæ, 3 to 5 μ wide.

Asci oblong to ovoid, 50 to 60 μ long, 20 μ in diameter. Spores oblong, rounded at the apices, 1-septate, constricted at the middle, brown, 20 μ long, 5 μ in diameter, upper cell the larger.

LUZON, Rizal Province, Antipolo, *Bur. Sci. 22700 M. Ramos*, June 14, 1915, on leaves of *Eugenia* sp., *Bur. Sci. 25070 H. S. Yates and M. Ramos*, September 23, 1915: Batangas Province, Pitong Sanay, *Bur. Sci. 22678 M. Ramos and D. Deroy* (type in Bur. of Sci. herb.), on leaves of *Eugenia* sp.

6. PARASTERINA FAGARAE (Yates) comb. nov. Plate 6, figs. 1 to 5.

Asterina fagarae YATES in Philip. Journ. Sci. Bot. 13 (1918) 373.

Fungous colony epiphyllous, forming round to irregular, black spots, 4 to 7 millimeters wide, sometimes running together and covering the whole surface of the leaf. Mycelium radiating, composed of a few, loose hyphæ, amber to dark brown, septate, 4 μ wide; branches effused, anastomosing. Hyphopodia few, generally alternate, although sometimes irregular, long, cylindrical, 13 to 16 μ long, 4 to 6 μ wide. Perithecia 125 to 150 μ in diameter.

Asci ovoid, 50 to 56 μ long, 36 to 39 μ in diameter. Spores heaped together, oblong, rounded at both ends, 1-septate, constricted at the middle, hyaline when young, brown when mature, smooth, 21 to 25 μ long, 11 to 12 μ in diameter.

LUZON, Rizal Province, *Bur. Sci. 26762 M. Ramos* (type in Bur. of Sci. herb.), October-November, 1916, on leaves of *Fagara avicennae* (= *Zanthoxylum avicennae*).

7. PARASTERINA HOMALOMENAE sp. nov. Plate 7, figs. 1 to 5.

Colonia fungosa, epiphylla, maculas parvas, rotundas, usque ad 8 mm. diam. plus minusve aequabiliter per folii superficiem sparsas efformans; mycelio sat abundanti ex hyphis sinuosis, sucineis vel fuscis, 4 ad 6 μ latis composito; hyphopodiis numerosis, lobatis, generatim oppositis, nonnumquam alternatis, 6 ad 12 μ longis, 5 ad 9 μ latis; peritheciis haud affluentibus, generatim cylindraceis sed saepenumero globosis, 180 ad 223 μ longis, 120 ad 403 μ latis; quoad corticem dehiscentibus, ex hyphis sinuosis compositis, 3 ad 4 μ latis; ambitu fimbriatis; ascis generatim globosis sed nonnumquam ovoideis, 29 ad 31 μ longis, 22 ad 26 μ in diametro; sporidiis oblongo-ellipsoideis,

1-septatis, medio constrictis laevibus, fuscis 14 ad 20 μ longis, 8 ad 11 μ in diametro; loculo superiore multo majore.

Fungous colony epiphyllous, forming small round spots up to 8 millimeters in diameter, more or less uniformly scattered on the surface of the leaf. Mycelium rather abundant, composed of sinuous hyphæ, amber to dark brown, 4 to 6 μ wide. Hyphopodia numerous, lobed, generally opposite, occasionally alternate, 6 to 12 μ long, 5 to 9 μ wide. Perithecia not abundant, commonly cylindrical but often globose, 180 to 223 μ long, 120 to 403 μ in diameter, walls splitting, composed of sinuous hyphæ, 3 to 4 μ wide; periphery fringed.

Asci usually globose, sometimes ovoid, 29 to 31 μ long, 22 to 26 μ in diameter. Spores oblong-ellipsoid, 2-celled, constricted at the middle, smooth, brown, 14 to 20 μ long, 8 to 11 μ in diameter, superior cell very much larger.

PANAY, Capiz Province, Jamindan, *Bur. Sci. 32104 M. Ramos* (type in Bur. of Sci. herb.), April 30, 1918, on leaves of *Homalomena philippinensis*.

This fungus is allied to *Parasterina spinosa* in the characters of the hyphopodia. The hyphopodia of the two fungi are 1-celled, distinctly lobed (with lobes that are identical in shape), and have no definite arrangement. However, the main difference between the two fungi is that *Parasterina spinosa* has spiny spores, while *P. homalomenae* has smooth spores.

8. PARASTERINA JASMINICOLA (Yates) comb. nov. Plate 8, figs. 1 to 6.

Asterina jasminicola YATES in Philip. Journ. Sci. Bot. 13 (1918) 373.

Fungous colony on both surfaces of the leaf, forming irregular spots 2 to 6 millimeters in diameter, often more or less distributed over the surface of the leaf. Mycelium composed of flexous, brown hyphæ, 4 to 5 μ wide. Hyphopodia lobed, numerous, 2-celled, irregularly distributed, never opposite; superior cell 5 to 7 μ long, 4 to 5 μ wide. Perithecia numerous, globose, black, opaque, dehiscent, 95 to 120 μ in diameter.

Asci globose, 22 to 26 μ in diameter. Spores oblong-ellipsoid, 1-septate, constricted at the single septum, brown, rounded at both ends, warted, 17 to 19 μ long, 8 to 10 μ in diameter.

LUZON, Ilocos Norte Province, Burgos, *Bur. Sci. 27797 M. Ramos* (type in Bur. of Sci. herb.), March 1, 1917, on leaves of *Jasminum* sp.: Bataan Province, Lamao, *Bur. Sci. 23991 M. Ramos*, December 27, 1915, on *Jasminum* sp.

9. PARASTERINA LITSEAE (Yates) comb. nov. Plate 9, figs. 1 to 6.

Asterina litseae YATES in Philip. Journ. Sci. Bot. 13 (1918) 373.

Amphigenous, although more abundant on the upper surface of the leaf, producing irregular patches, more or less covering the whole surface. Mycelium effused, loose, irregular, radiating, consisting of dark brown hyphæ, 3.5 to 4.5 μ wide; branches anastomosing. Hyphopodia few, round to lobed, 1-celled, 7 to 8 μ long, 3 to 5 μ wide, arranged irregularly. Perithecia very numerous, oftentimes in clusters, cylindrical, occasionally round, 100 to 140 μ in diameter; walls splitting, composed of brown hyphæ, 2 to 3 μ wide, which radiate from the ostiole; periphery fringed, with short radiating hyphæ.

Asci ovoid to globose, 28 to 30 μ long, 18 to 24 μ in diameter. Spores ellipsoid, rounded at both ends, 1-septate, constricted at the middle, brown, smooth, 15 to 17 μ long, 8 to 9 μ in diameter.

LUZON, Ilocos Norte Province, Burgos, *Bur. Sci. 27842 M. Ramos* (type in Bur. of Sci. herb.), March 12, 1917, on leaves of *Litsea* sp.; Bangui, *Bur. Sci. 27775 M. Ramos*, February 25, 1917, on leaves of *Litsea* sp.

10. PARASTERINA MOMORDICAE (Yates) comb. nov.

Asterina momordicae YATES in Philip. Journ. Sci. Bot. 13 (1918) 374.

Epiphyllous, spots decidedly black, 2 to 4 millimeters broad. Mycelium composed of dark brown, distantly septate hyphæ, 5 to 6 μ wide. Hyphopodia lobed, numerous, irregular, unicellular, 8 to 12 μ long. Perithecia rounded, 80 to 90 μ in diameter, dehiscent, composed of straight, brown, radiating hyphæ, 2.5 to 3 μ wide.

Asci subglobose, 35 to 40 μ long, 22 to 24 μ in diameter. Spores oblong, rounded at both ends, 1-septate, constricted at the middle, brown, smooth, 19 to 21 μ long, 9 to 11 μ in diameter, superior cell the larger.

LUZON, Tayabas Province, Mount Binuang, *Bur. Sci. 28890 M. Ramos and G. Edaño*, May 13, 1917, on leaves of *Momordica* sp.

The above description is the translation of the original Latin diagnosis. I have been unable to find the type specimen.

11. PARASTERINA NYCTICALIAE (Yates) comb. nov. Plate 10, figs. 1 to 5.

Asterina nycticaliae YATES in Philip. Journ. Sci. Bot. 12 (1917) 371.

Spots mostly epiphyllous, rounded to irregular, black, 3 to 5 millimeters in diameter. Mycelium composed of a few, dark

brown, septate hyphæ, 5 to 6 μ wide; branches anastomosing, generally opposite. Hyphopodia 1-celled, numerous, often alternate to irregular, flabellilobed, 10 to 11 μ long, 12 to 15 μ wide. Perithecia numerous, globose, flattened, small, 90 to 110 μ in diameter, dark brown, slightly opaque, splitting, composed of radiating hyphæ, 4 to 5 μ wide.

Asci ovoid to subglobose, 8-spored, 26 to 28 μ long, 18 to 20 μ in diameter. Spores oblong, rounded at both ends, 1-septate, constricted at the middle, brown, smooth, 15 to 18 μ long, 7 to 8 μ in diameter.

LUZON, Camarines Norte Province, Basud, *Bur. Sci. 25669 H. S. Yates* (type in Bur. of Sci. herb.), December 11, 1916, on leaves of *Nycticalos cuspidatum;* Paracale, *Bur. Sci. 34018 M. Ramos and G. Edaño,* December 22, 1918, on leaves of *Wrightia* sp.

12. **PARASTERINA PEMPHIDIOIDES** (Cooke) Theissen. Plate 11, figs. 1 to 4.

Parasterina pemphidioides (Cooke) THEISSEN in Ann. Mycol. 17 (1918) 246.

Forming amphigenous, black, small, irregularly circular spots 5 to 8 millimeters in diameter, soon coalescing, sometimes almost covering the surface of the leaf. Mycelium floccose, brown, laterally branching, anastomosing, composed of regularly septate hyphæ, 4 to 6 μ wide. Hyphopodia fairly numerous, 2-celled, alternate to irregularly 1-sided, truncate to nearly rounded at the end, 9 to 17 μ long, 6 to 9 μ wide. Perithecia numerous, shiny, hemispherical, rounded to long cylindrical, 100 to 300 μ diameter, splitting, composed of amber-colored hyphæ, 3 to 4 μ wide.

Asci obovoid to almost rounded, 35 to 41 μ long, 25 to 30 μ in diameter. Spores ellipsoid, constricted at the single septum, brown, banded, superior cell the larger, 25 to 30 μ long, 9 to 12 μ in diameter.

LUZON, Ilocos Norte Province, Burgos, *Bur. Sci. 27833 M. Ramos,* March 15, 1917, on *Eugenia jambolana:* Benguet Subprovince, *Bur. Sci. 32091 J. K. Santos,* June 9, 1918, on *Eugenia* sp.: Isabela Province, Palanan Bay, *Sydow Fungi exotici exsicati 270 L. Escritor,* June, 1913, on *Eugenia subrotundifolia:* Tayabas Province, Guinayangan, *Bur. Sci. 20924 E. D. Merrill,* May, 1913, on *Eugenia* sp.: Bataan Province, Mount Mariveles, *Bur. Sci. 19075 P. W. Graff,* November, 1912, on *Eugenia* sp.: Rizal Province, *Bur. Sci. 23951 M. Ramos,* December 14, 1915, on *Eugenia calubcob;* Morong, *Bur. Sci. 23892 M. Ramos,* November 23, 1915, on *Eugenia jambolana:* Camarines Norte

Province, Paracale, *Bur. Sci. 33972 M. Ramos and G. Edaño* December 11, 1918, on *Shorea* sp. PALAWAN, Mount Capoas, *Bur. Sci. 9082 E. D. Merrill,* April 21, 1913; Taytay, *Bur. Sci. 8738, 8788 E. D. Merrill,* April 10, 1913, on *Eugenia* sp. BUSUANGA, Concepcion, *Bur. Sci. 41443 M. Ramos,* September 8, 1922, on *Eugenia.* SULU ARCHIPELAGO, Bengas Island, *Bur. Sci. 36158 H. S. Yates,* October 27, 1919.

The above description is taken from a topotype (*Bur. Sci. 21902 M. Ramos*), collected near Antipolo, Rizal Province, August, 1915, on *Eugenia jambolana,* which according to Theissen [6] agrees closely with the type specimen in having large, banded spores.

This species is allied to *Parasterina incisa,*[7] of India, but differs in its smaller spores, and to *Parasterina japonica* Theiss.,[8] of Japan, but differs in having larger asci, and shorter spores, which are not banded.

13. PARASTERINA RAMOSII Sydow. Plate 12, figs. 1 to 5.

Parasterina ramosii SYDOW in Ann. Mycol. 14 (1917) 246.

Epiphyllous, spots at first small, soon coalescing into irregular patches. Mycelium composed of rectangular, chestnut brown hyphæ, 14 to 30 μ long, 5 to 7 μ wide, branching at right angles. Hyphopodia rather numerous, usually, but not constantly opposite, 2-celled, 12 to 18 μ long, 6 to 8 μ wide, cylindrical, straight, not lobed, base cell small. Perithecia at first 200 to 300 μ in diameter, becoming oblong-elliptic and up to 600 μ long, 200 to 300 μ wide, margin fringed, radiating, opaque, stellate, dehiscent.

Asci ellipsoid to ovoid, 50 to 80 μ long, 35 to 45 μ in diameter. Spores dark brown when mature, opaque, 26 to 30 μ long, 12 to 14 μ in diameter.

LUZON, Rizal Province, Antipolo, *Bur. Sci. 23906 M. Ramos* (cotype in Bur. of Sci. herb.), *23923,* November 24, 1915, on *Eugenia jambolana.*

Parasterina ramosii resembles somewhat *P. pemphidioides,* but differs in its opposite hyphopodia and bandless spores. It is allied to *Parasterina japonica* Theiss.,[9] of Japan, but differs in having smaller asci.

[6] Ann. Mycol. 15 (1917) 246.

[7] Ann. Mycol. 9 (1911) 390.

[8] Gatt. Asterina, In Abh. der K. K. Zoölogisch-Botanischen Gesellshaft (1913) 43, tab. VI, figs. 22–23 et VIII figs. 8, 13, 14.

[9] Gatt. Asterina (1913) 43, tab. VI figs. 22–23 and VIII figs. 8, 13, 14.

14. PARASTERINA SAMARENSIS nom. nov. Plate 13, figs. 1 to 5.

Asterina ramosii YATES in Philip. Journ. Sci. Bot. 13 (1918) 373.

Hypophyllous; spots circular, dark gray, 4 to 10 millimeters in diameter. Mycelium composed of effused, loose, brown, branching, septate hyphæ, 4 to 5 μ wide. Hyphopodia few, alternate to irregular, lobed, 6 to 8 μ long, 5 μ wide. Perithecia numerous, round, subopaque, 80 to 120 μ in diameter, stellate, dehiscent, composed of radiating hyphæ, 4 to 5 μ wide.

Asci ovoid to globose, 18 to 20 μ long, 15 to 17 μ in diameter. Spores conglobate, oblong, sometimes broad, rounded at both ends, 1-septate, constricted at the middle, 14 to 16 μ long, 6 to 8 μ in diameter.

LUZON, Camarines Norte Province, Paracale, *Bur. Sci. 33955 M. Ramos and G. Edaño*, December 18, 1918, on *Dillenia philippinensis*. SAMAR, Catubig River, *Bur. Sci. 24643* (type in Bur. of Sci. herb.), *24637 M. Ramos*, February 20, 1916, on *Dillenia philippinensis*.

The transfer of this fungus to the new genus *Parasterina*, makes its name *Parasterina ramosii*, but, unfortunately another species has been named *Parasterina ramosii*. It is, therefore, necessary to give the Yates fungus another name, and *Parasterina samarensis* is proposed.

Parasterina nycticaliae (Yates) agrees with *P. samarensis* in having identical perithecia, ovoid to globose asci, and oblong spores. It differs, however, in having somewhat smaller spores.

15. PARASTERINA SPINOSA sp. nov. Plate 14, figs. 1 to 5.

Colonia fungosa stricte epiphylla, maculas rotundas, atras, elevatas, 0.5 ad 5 mm latas, dense et sine ordine per folii superficiem sparsas efformans; mycelio exiguo, inter-texto, ex hyphis flavis vel sucineis, densis, septatis, 3 ad 5 μ latis; ramis raris, leviter anastomosantibus; hyphopodiis sat abundantibus, sine ordine dispositis, distincte lobatis; peritheciis affluentibus, globosis, 95 ad 172 μ diam. cortice fusco parte interiore caeruleo-virescente, contextu stellatim facile dehiscentibus, ex hyphis septatis composito, 2 ad 4 μ latis, ambitu nonfimbriatis; ascis sat numerosis, generatim globosis, nonnumquam subglobosis vel ovoideo-globosis, 35 ad 46 μ longis, 29 ad 31 μ in diametro, sporidiis tenuiter spinosis, 1-septatis, medio constrictis, utrinque rotundatis, 22 ad 25 μ longis, 9 ad 10 μ latis, loculo superiore majore.

Fungous colony strictly epiphyllous, forming round, black, elevated spots, ranging from 0.5 millimeter to about 5 milli-

meters wide, scattered thickly and irregularly over the surface of the leaf. Mycelium sparse, intertwining, composed of straw to beautiful amber-colored, compact, septate hyphæ, 3 to 5 μ wide, branches rare, slightly anastomosing. Hyphopodia abundant, with no definite arrangement, distinctly lobed, stipitate, 9.5 to 11 μ long, 6.5 to 10 μ wide. Perithecia rather abundant, globose, 95 to 172 μ in diameter, outside coating dark brown, inside layer bluish green; wall splitting, composed of septate hyphæ, 2 to 4 μ wide. Periphery not fringed.

Asci quite numerous, usually globose, sometimes subglobose or ovoid-globose, 35 to 46 μ long, 29 to 31 μ in diameter. Spores with slender spines, 2-celled, constricted at the single septum, rounded at both ends, 22 to 23 μ long, 9 to 10 μ in diameter. superior cell the larger.

LUZON, Tayabas Province, Mount Bunuan, *Bur. Sci. 28884 M. Ramos* (type in Bur. of Sci. herb.), May 19, 1919, on *Cissus*.

Parasterina spinosa resembles *Parasterina tayabensis* in having spores that are spiny; however, those of the former are slenderer. In the hyphopodia there is a close similarity with those of *Parasterina homalomenae*. The specific name, *spinosa*, has reference to the spiny character of the spores.

16. PARASTERINA TAYABENSIS (Yates) comb. nov. Plate 15, figs. 1 to 6.

Asterina tayabensis YATES in Philip. Journ. Sci. Bot. 12 (1917) 372.

Spots epiphyllous, rounded, 3 to 4 millimeters wide, finally coalescing and covering large portions of the leaf surface. Mycelium abundant, composed of brown, anastomosing, septate hyphæ, 10 to 12 μ long, 5 to 7 μ wide; branches irregular. Hyphopodia few, scattered, globose to short cylindrical, rounded at the end, one-celled, 10 to 12 μ long, 5 to 7 μ wide. Perithecia numerous, 130 to 200 μ in diameter, slender, stellate, dehiscent, radiating, composed of hyphæ, 2 to 6 μ wide.

Asci subglobose, 30 μ long, 25 μ in diameter. Spores oblong, rounded at both ends, 1-septate, constricted at the middle, with coarse spines, 22 μ long, 10 μ in diameter.

LUZON, Camarines Norte Province, Basud, *Bur. Sci. 25635 M. Ramos* (type in Bur. of Sci. herb.), December 19, 1916, on unknown host.

In Yates's description the specimen is labelled Basiad, Tayabas, but the field label states that the specimen was collected in Basud, Camarines Norte.

Host index to the species.

Host.	Fungus.
Astronia sp. (Melastomataceæ).	*Parasterina astroniae.*
Canthium sp. (Rubiaceæ).	*Parasterina canthi.*
Cissus (Vitaceæ).	*Parasterina spinosa.*
Cipadessa baccifera (Meliaceæ).	*Parasterina cipadessae.*
Dillenia sp. (Dilleniaceæ).	*Parasterina dilleniae.*
Dillenia sp. (Dilleniaceæ).	*Parasterina samarensis.*
Dillenia philippinensis (Dilleniaceæ).	*Parasterina samarensis.*
Eugenia calubcob (Myrtaceæ).	*Parasterina pemphidioides.*
Eugenia jambolana (Myrtaceæ).	*Parasterina pemphidioides.*
Eugenia subrotundifolia (Myrtaceæ).	*Parasterina pemphidioides.*
Eugenia sp. (Myrtaceæ).	*Parasterina eugeniae.*
Eugenia sp. (Myrtaceæ).	*Parasterina pemphidioides.*
Eugenia sp. (Myrtaceæ).	*Parasterina ramosii.*
Fagara avicennae=*Zanthoxylum avicennae* (Rubiaceæ).	*Parasterina fagarae.*
Homalomena philippinensis (Araceæ).	*Parasterina homalomenae.*
Jasminum sp. (Oleaceæ).	*Parasterina jasminicola.*
Litsea sp. (Lauraceæ).	*Parasterina litseae.*
Momordica sp. (Cucurbitaceæ).	*Parasterina momordicae.*
Nycticalos cuspidatum (Bignoniaceæ).	*Parasterina nycticaliae.*
Shorea sp. (Dipterocarpaceæ).	*Parasterina pemphidioides.*
Wrightia (Apocinaceæ).	*Parasterina nycticaliae.*
Unknown host.	*Parasterina tayabensis.*

ACKNOWLEDGMENTS

The writer acknowledges his obligations to Dr. C. J. Humphrey, mycologist in charge of investigations in mycology and plant pathology, Bureau of Science, for reviewing the work; to Rev. Dr. Henry C. Avery, S. J., of the Ateneo de Manila, for translating the English diagnoses into Latin; to Dr. G. O. Ocfemia, of the College of Agriculture, University of the Philippines, Los Baños, and to the Director of the Botanic Garden, Buitenzorg, Java, for the loan of specimens of the family Microthyriaceæ.

ILLUSTRATIONS

PLATE 1. PARASTERINA ASTRONIAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a leaf surface showing fungous colony; × 20.
2. Perithecium, with mycelium; × 254.
3. An ascus, with paraphyses and ascospores; × 1431.
4. Two ascospores; × 1431.
5. Mycelium, with hyphopodia; × 1431.

PLATE 2. PARASTERINA CANTHI (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a leaf surface showing fungous colony; × 20.
2. Perithecium, with mycelium and hyphopodia; × 254.
3. Ascus, with ascospores and paraphyses; × 1431.
4. Two ascospores; × 1431.
5. Mycelium, with hyphopodia and a developing perithecium; × 1431.

PLATE 3. PARASTERINA CIPADESSAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of fungous colony on the leaf surface; × 20.
2. Perithecium, with mycelium; × 254.
3. Ascus, with paraphyses and ascospores; × 1431.
4. Two ascospores; × 1431.
5. Mycelium, with hyphopodia; × 1431.

PLATE 4. PARASTERINA DILLENIAE (SYDOW) COMB. NOV.

FIG. 1. Photomicrograph of fungous colony on the leaf surface; × 20.
2. Perithecium; × 254.
3. Portion of perithecium showing perithecial hyphæ in detail; × 1431.
4. Asci, showing two types, *a* and *b*, with paraphyses and ascospores; × 1431.
5. Two warted ascospores; × 1431.
6. Mycelium, with one-celled hyphopodia; × 1431.

PLATE 5. PARASTERINA EUGENIAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony on the leaf surface; × 20.
2. Perithecium; × 254.
3. Asci, *a* and *b*, with paraphyses and ascospores; × 1431.
4. Two ascospores.
5. Mycelium, with one-celled hyphopodia.

PLATE 6. PARASTERINA FAGARAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia on the leaf surface; × 20.
2. Perithecium, with mycelium; × 1431.
3. Ascus, with ascospores and paraphyses; × 1431.
4. Three ascospores; × 1431.
5. Mycelium, with long, cylindrical, one-celled hyphopodia; × 1431.

PLATE 7. PARASTERINA HOMALOMENAE SP. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia on the leaf surface; × 20.
2. Portion of perithecium, showing wall structure; × 1431.
3. Asci, *a* and *b*, with paraphyses and ascospores; × 1431.
4. Three ascospores; × 1431.
5. Mycelium, with hyphopodia; × 1431.

PLATE 8. PARASTERINA JASMINICOLA (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia; × 20.
2. Perithecium; × 254.
3. Portion of a perithecium, showing wall structure; × 1431.
4. Ascus, with paraphyses and ascospores; × 1431.
5. Three warted ascospores; × 1431.
6. Mycelium, showing hyphopodia; × 1431.

PLATE 9. PARASTERINA LITSEAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia; × 20.
2. Perithecia, showing two types, *a* and *b*; × 254.
3. Portion of perithecium, showing wall structure; × 1431.
4. Asci, *a* and *b*, with paraphyses and ascospores; × 1431.
5. Three ascospores; × 1431.
6. Mycelium, with one-celled hyphopodia; × 1431.

PLATE 10. PARASTERINA NYCTICALIAE (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia; × 20.
2. Perithecia, with portion of mycelium.
3. Portion of mycelium, showing wall structure.
4. Two types of asci, *a* and *b*, with paraphyses and ascospores.
5. Mycelium, with two fork-lobed hyphopodia.

PLATE 11. PARASTERINA PEMPHIDIOIDES (COOKE) THEISSEN

FIG. 1. Photomicrograph of a fungous colony on the leaf surface, showing perithecia; × 20.
2. Perithecia, showing two types, *a* and *b*; × 254.
3. Two types of asci, *a* and *b*, with paraphyses and ascospores; × 1431.
4. Two ascospores, showing banded character; × 1431.

PLATE 12. PARASTERINA RAMOSII SYDOW

FIG. 1. Photomicrograph of a fungous colony, showing perithecia on the leaf surface; × 20.
2. Perithecia, showing two types, *a* and *b*; × 254.
3. Ascus, with paraphyses and ascospores; × 1431.
4. Two ascospores; × 1431.
5. Mycelium, with opposite, two-celled hyphopodia; × 1431.

PLATE 13. PARASTERINA SAMARENSIS NOM. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia; × 20.
2. Perithecium; × 254.
3. Two types of asci, *a* and *b*, with paraphyses and ascospores; × 1431.
4. Three ascospores; × 1431.
5. Mycelium, with many lobate hyphopodia; × 1431.

PLATE 14. PARASTERINA SPINOSA SP. NOV.

FIG. 1. Photomicrograph of a fungous colony, showing perithecia; × 20.
2. Portion of perithecium with mycelium; × 254.
3. Asci, *a* and *b*, with paraphyses and ascospores; × 1431.
4. Two spiny ascospores; × 1431.
5. Mycelium, with lobate hyphopodia; × 1431.

PLATE 15. PARASTERINA TAYABENSIS (YATES) COMB. NOV.

FIG. 1. Photomicrograph of a fungous colony on the leaf surface, showing the perithecia; × 20.
2. Perithecium, showing peculiarity of splitting; × 254.
3. Portion of perithecium, showing arrangement of hyphæ; × 1431.
4. Ascus, with paraphyses and ascospores; × 1431.
5. Three spiny ascospores; × 1431.
6. Mycelium, with one-celled hyphopodia; × 1431.

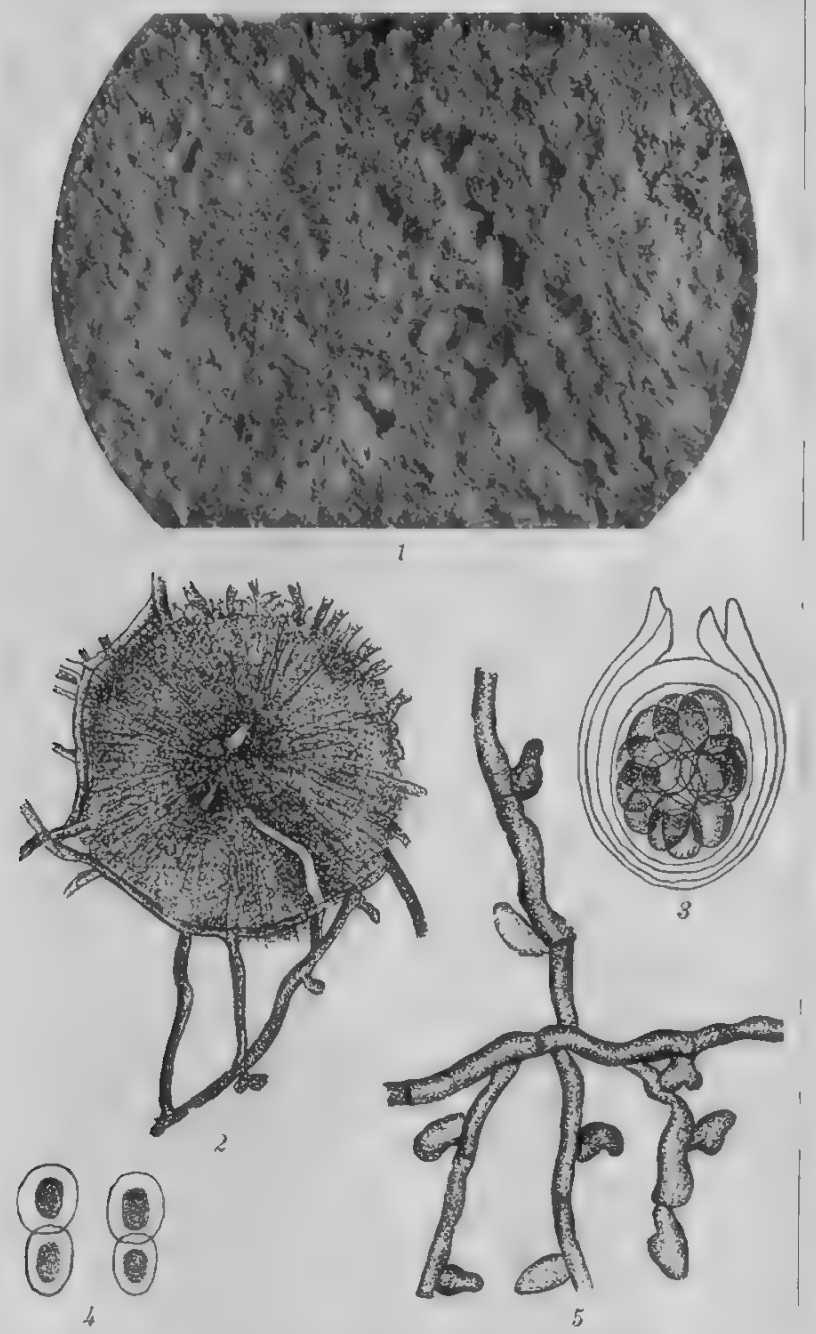


PLATE 1.

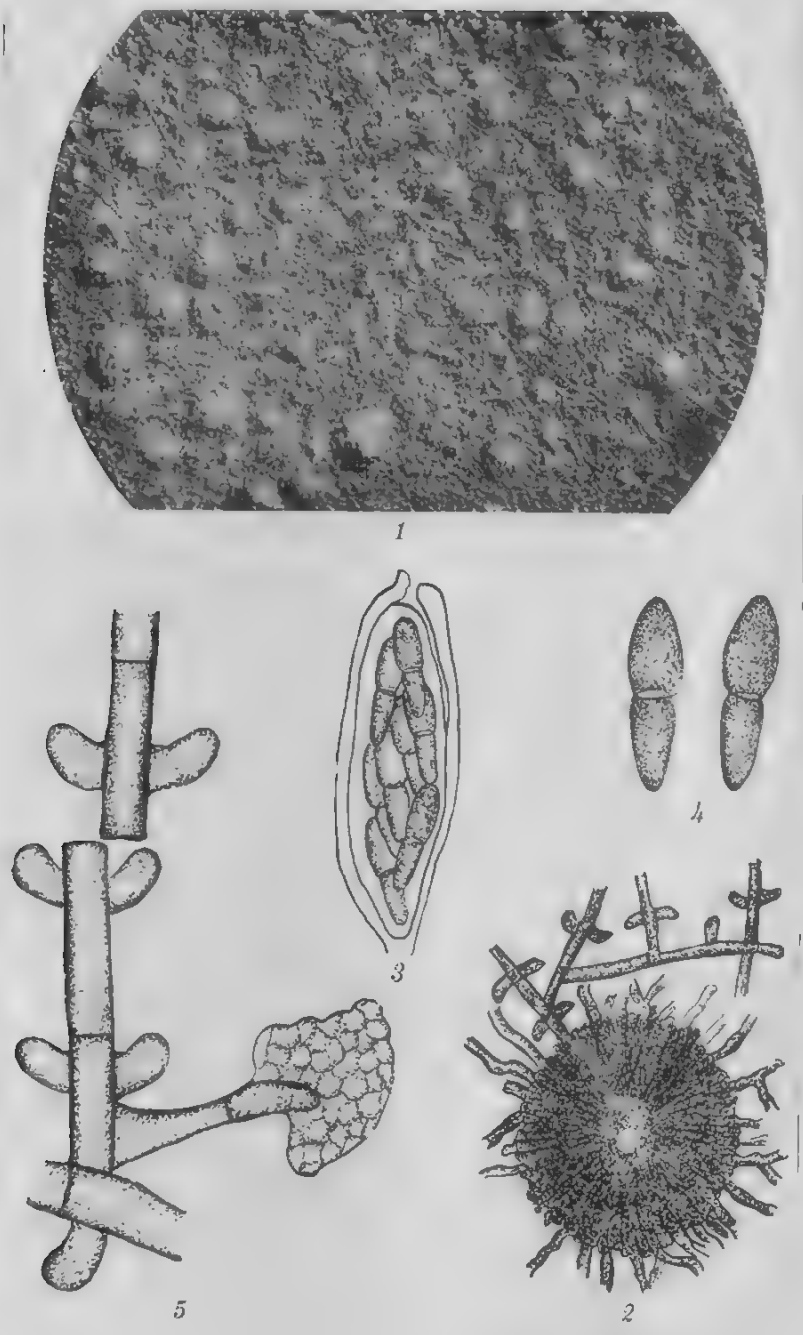
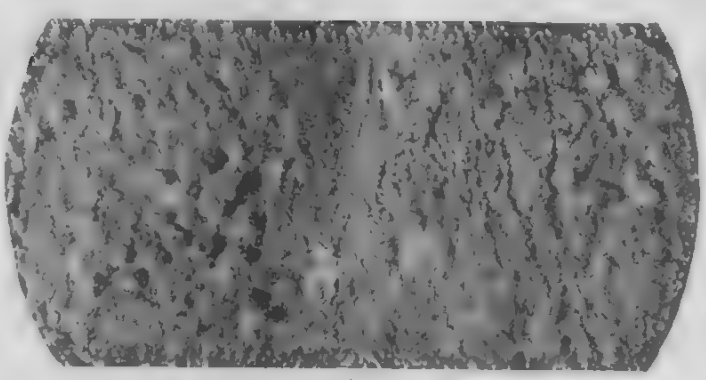
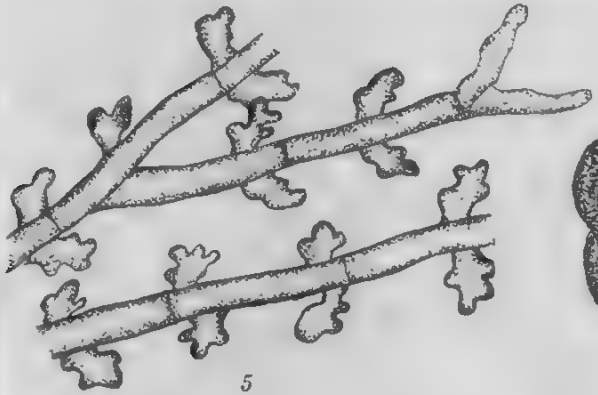


PLATE 2.

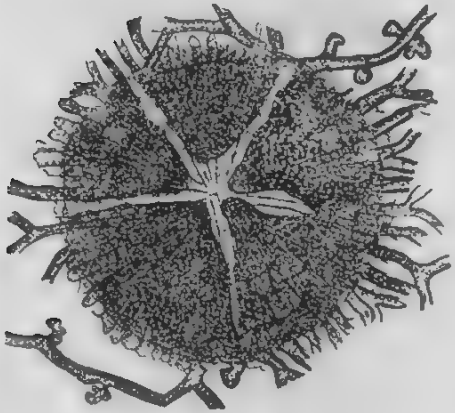


1

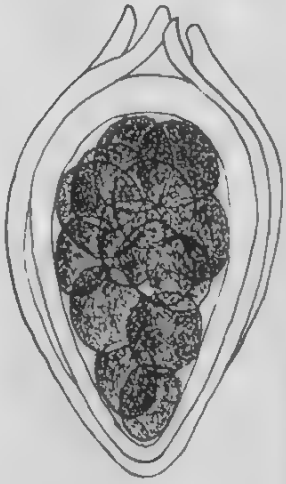


5

4



2



3

PLATE 3.

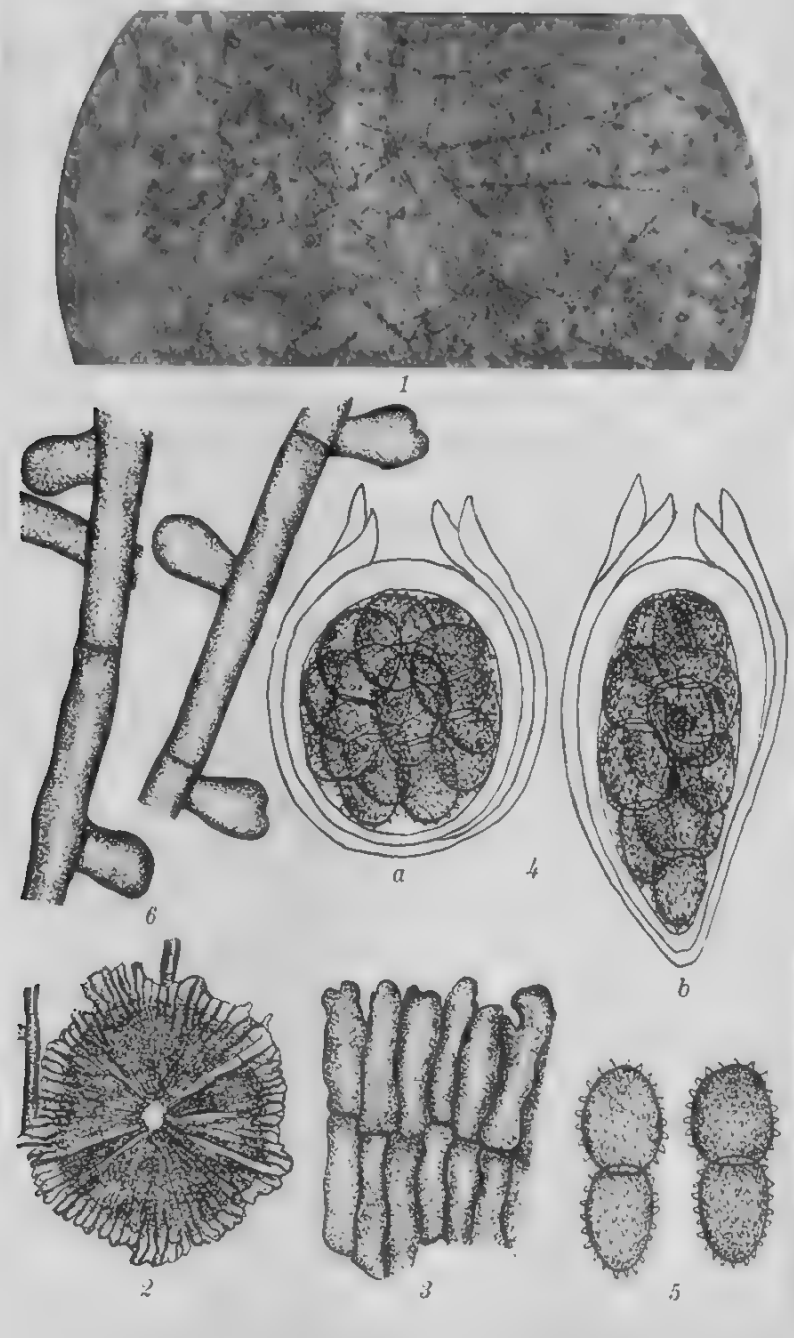


PLATE 4.

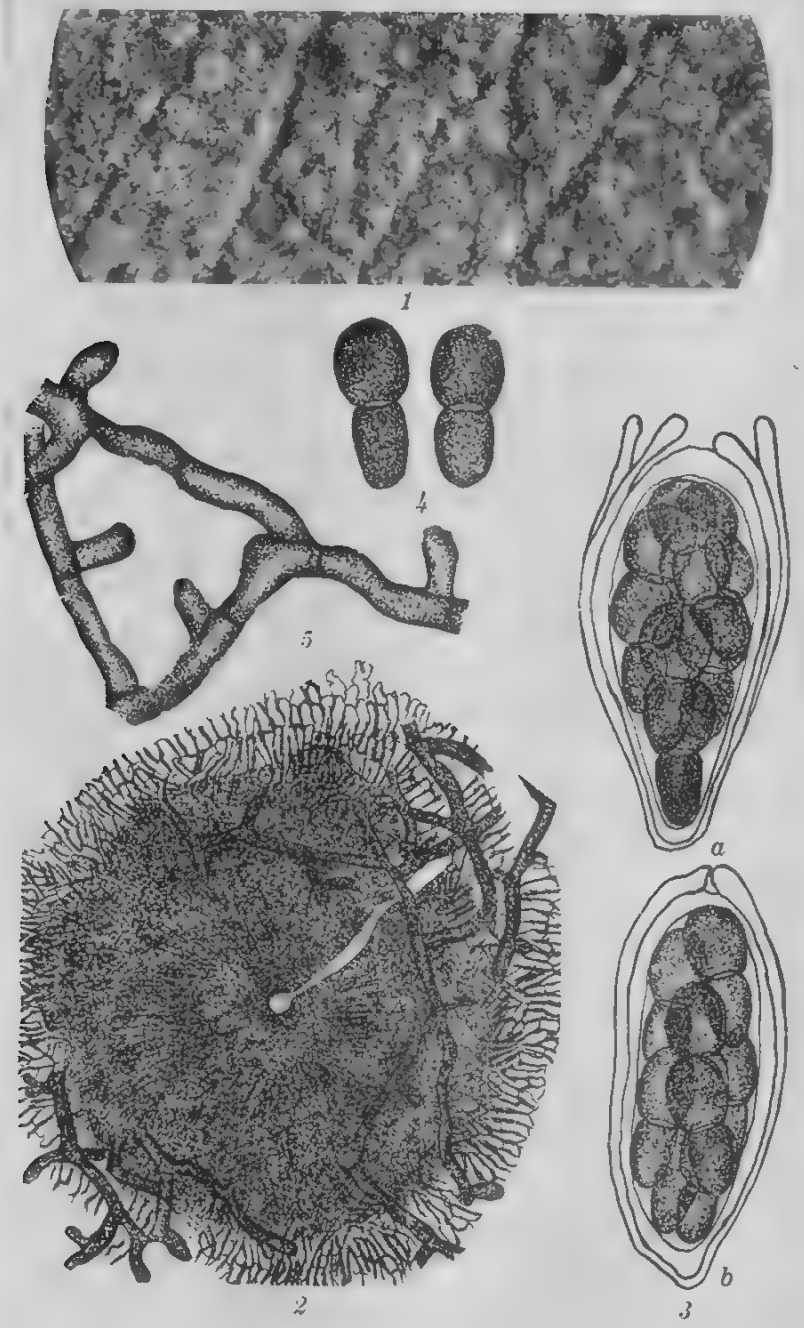


PLATE 5.

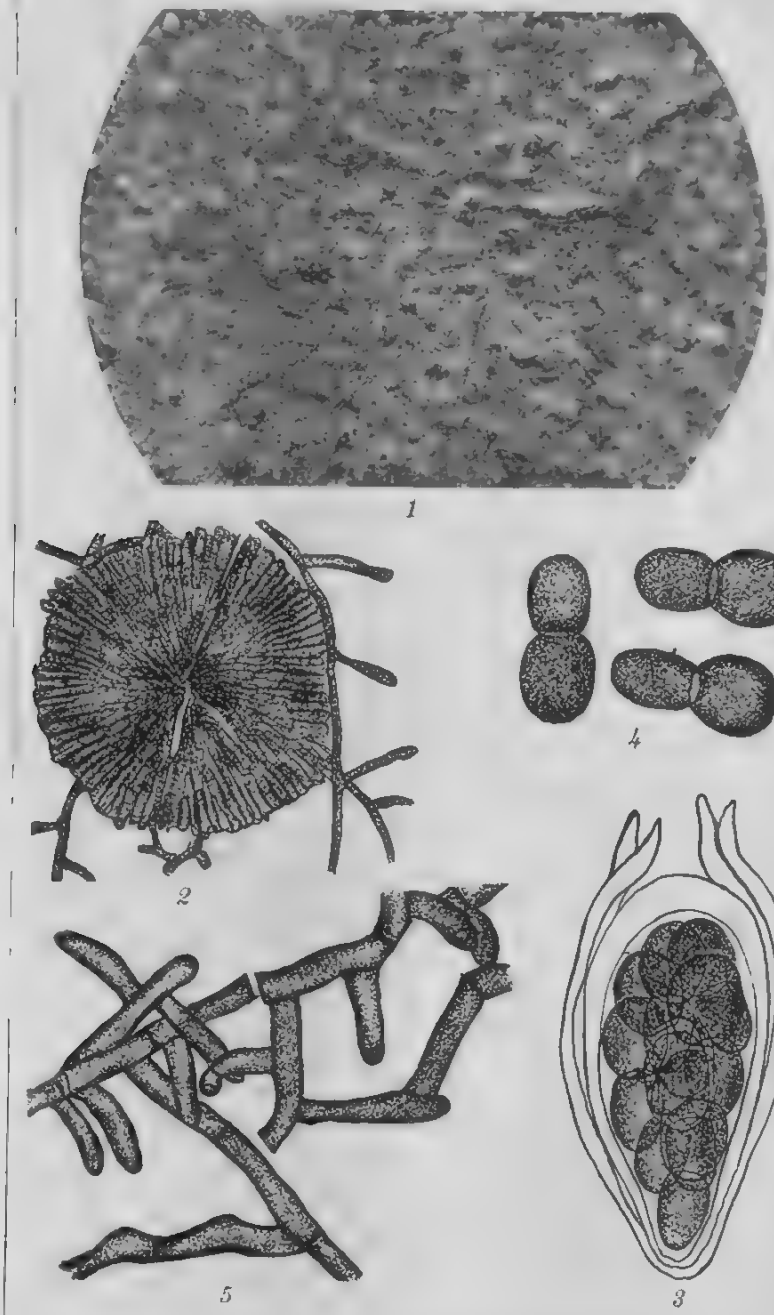


PLATE 6.

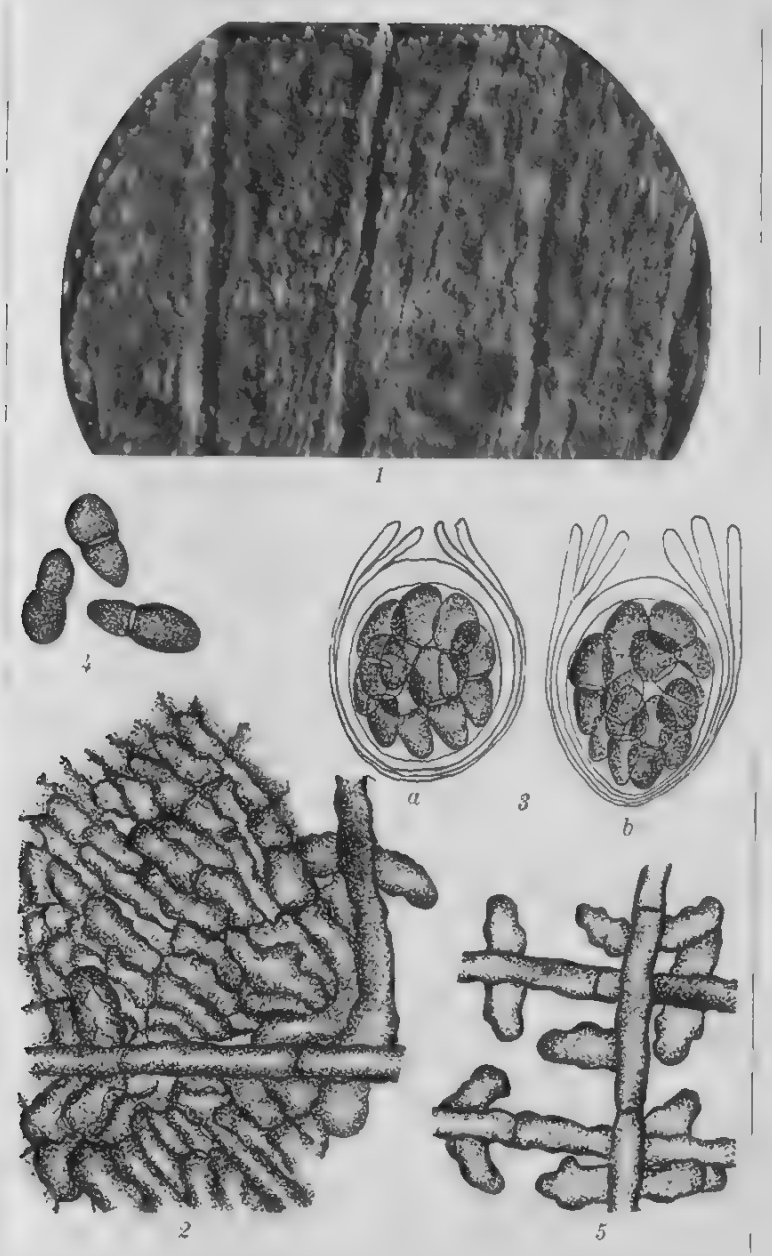


PLATE 7.

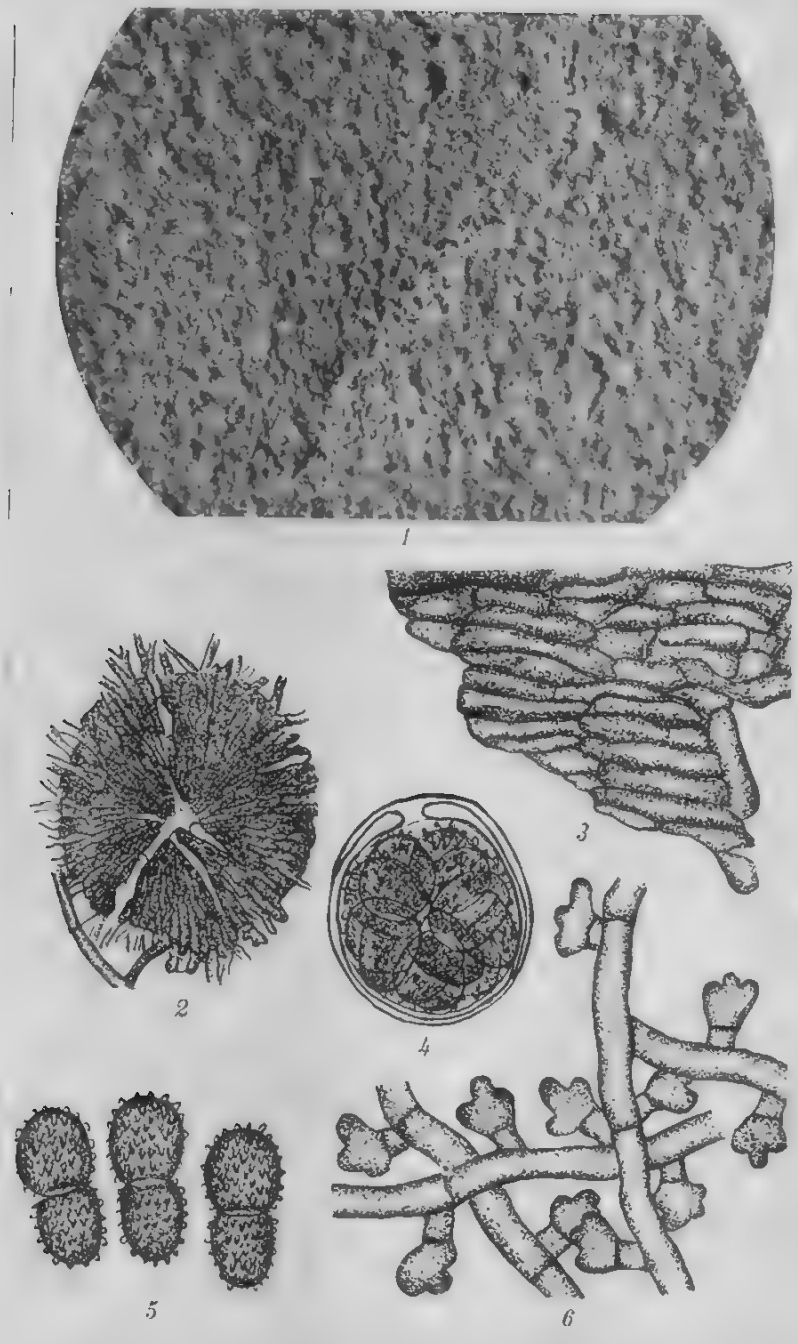


PLATE 8.

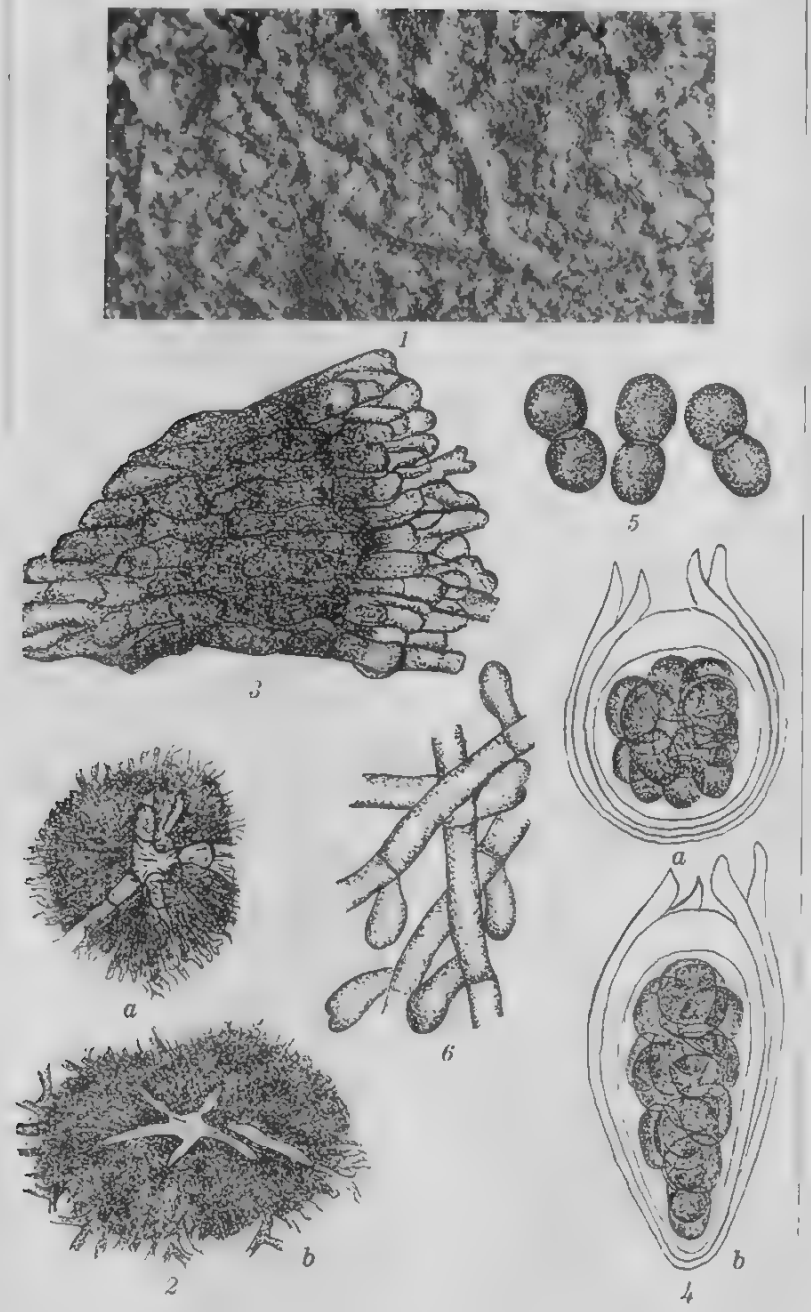


PLATE 9.

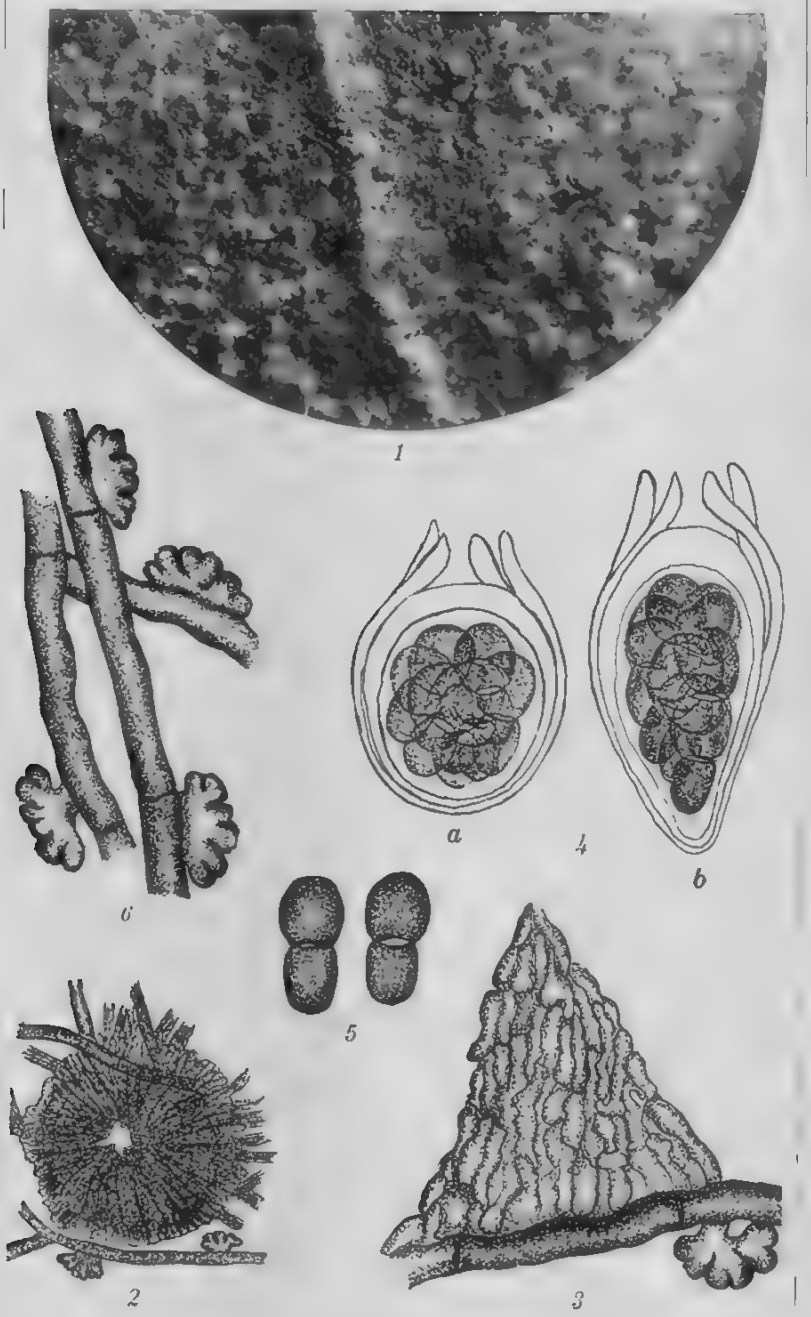


PLATE 10.

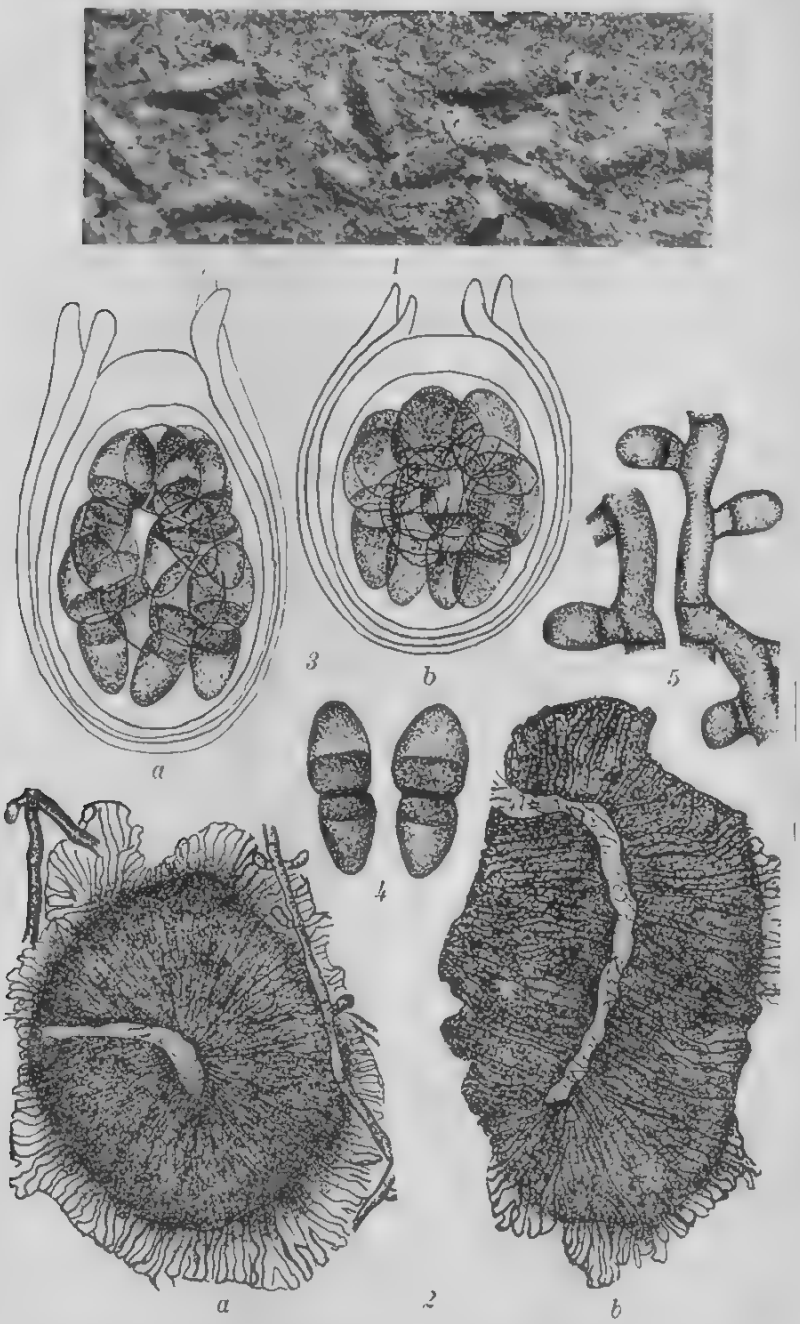


PLATE 11.

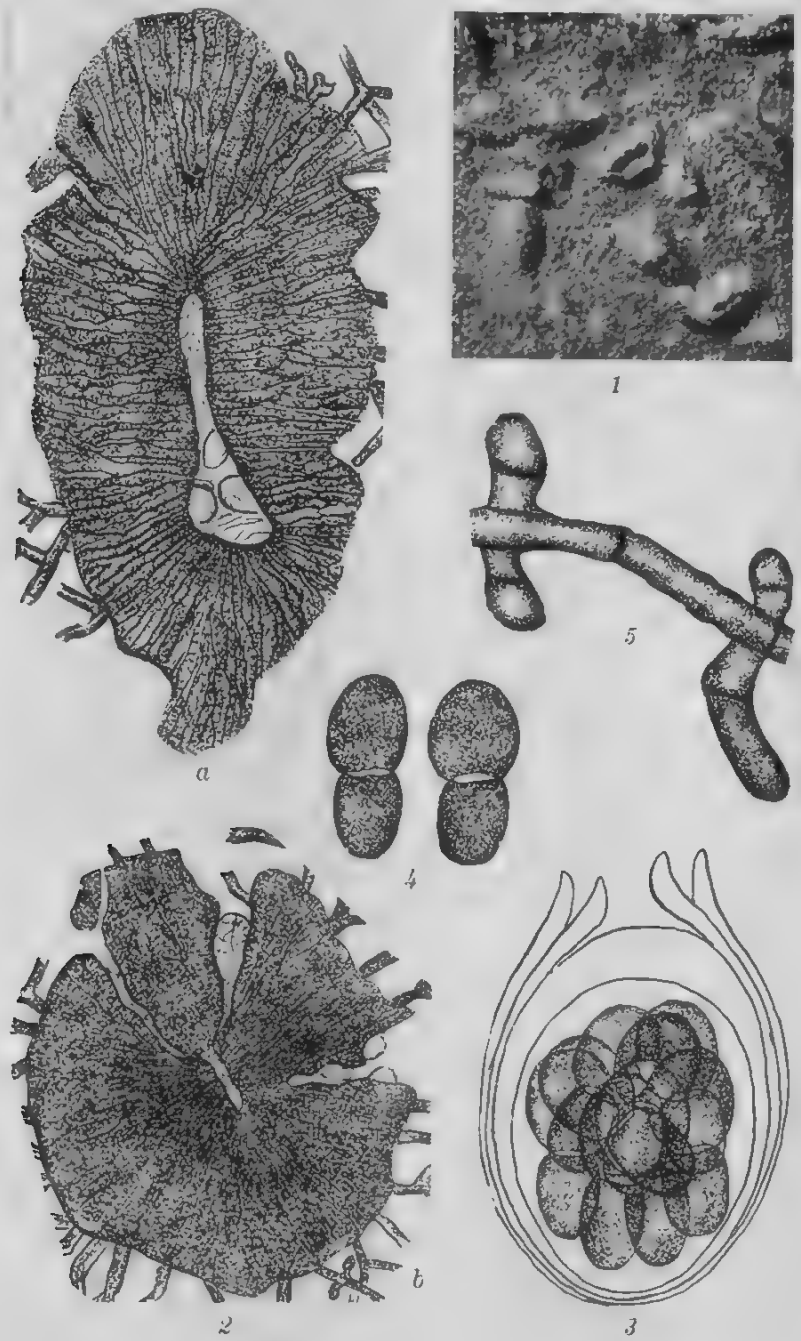
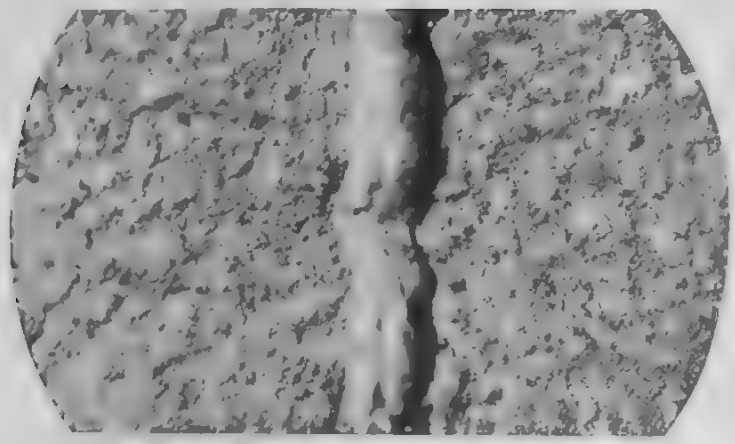
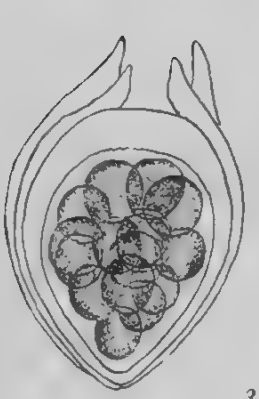


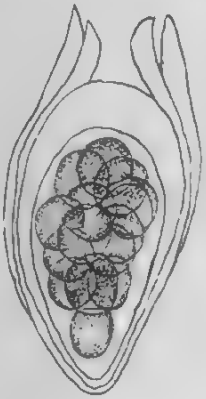
PLATE 12.



1

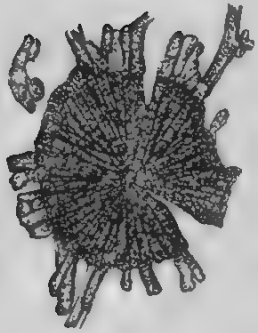


a



3 b

4



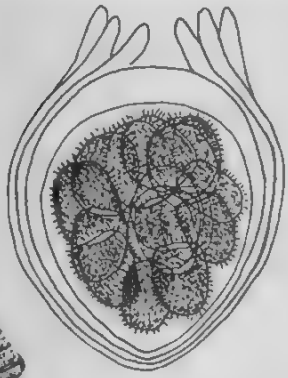
2

5

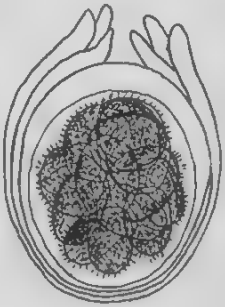
PLATE 13.

1

4



a

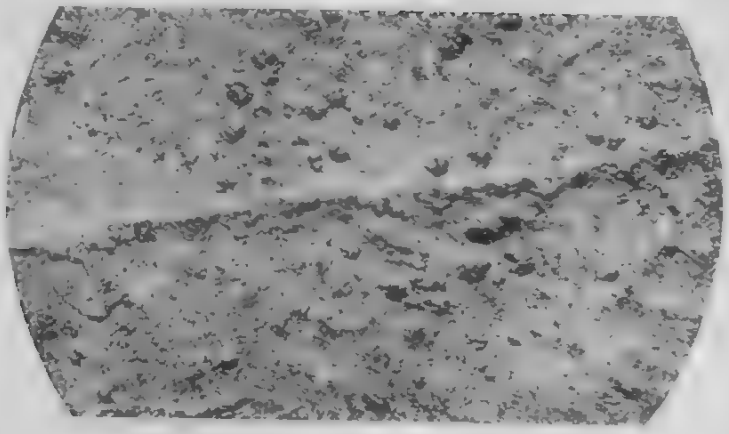


3 b

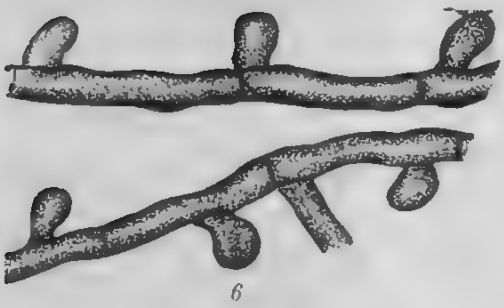
2

5

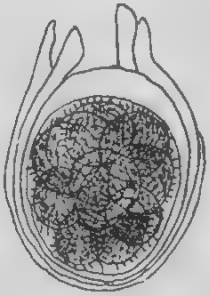
PLATE 14.



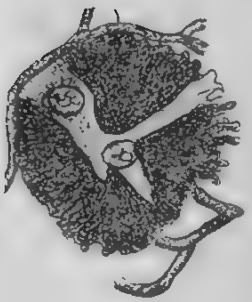
1



6

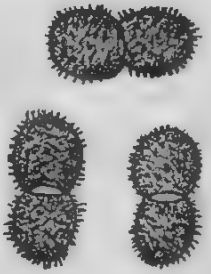


4



2

3



5

PLATE 15.

NITROGEN DISTRIBUTION IN THE LEAVES OF PHILIPPINE CAMPHOR TREES

By JOAQUIN MARAÑON

Of the Division of Botany, Bureau of Science, Manila

The partition of the nitrogenous constituents of plant and animal tissues is one of the means employed for the investigation of various kinds of biochemical problems. Primarily, it gives an insight into the composition of the protein which is the major constituent of protoplasm. It serves as an index to ascertain the influence of factors on the nitrogen metabolism of an organism. It is also useful in interpreting the results of the chemical analysis of foodstuffs.

Two years ago, West and Taguibao [1] carried out an investigation on the camphor content of camphor trees growing at Baguio in the Mountain Province of the Philippines. They found that camphor occurs almost entirely in the leaves of Philippine trees and, by distilling the leaves, crystal camphor containing a small amount of oil is usually obtained. They found, however, that when the leaves of a certain few trees were distilled there was obtained only a yellow volatile oil instead of the usual camphor crystals. This volatile oil has a negative rotation and is evidently quite different from the ordinary camphor oil which is dextrorotatory. Since the trees, from which this unusual lævo oil was obtained, were growing promiscuously among the ordinary camphor trees, it would seem unlikely that the production of this lævo oil was due to the ordinary environmental factors.

From a physiological point of view it seemed interesting to compare the nitrogen distribution in leaves from trees giving a lævo oil with that in leaves yielding the usual crystal camphor, and to ascertain if these two kinds of camphor trees (lævo oil and crystals) give different nitrogen partition products.

EXPERIMENTAL PROCEDURE

Collection of material.—The camphor trees selected for this investigation were a portion of those labelled by West and Taguibao for their work on Philippine camphor.

[1] Philip. Journ. Sci. 41 (1930) 103.

The leaves were collected from freshly cut branches. Care was taken that the collection of samples from the leaves of trees giving lævo oil and from those giving crystal camphor was done on the same day. The trees selected were growing side by side in the same locality. The samples were sorted into young, full-grown, and old leaves showing signs of chlorophyll degradation. These leaves were air-dried and reduced to a fine powder. Moisture determinations for each sample were made by heating the leaves to constant weight at a temperature of 105° C. in order to express the percentage of the nitrogenous constituents on a moisture- and camphor-free basis.

Methods of analysis.—The total nitrogen was determined directly from the air-dried samples of the leaves according to the official Gunning Method, modified to include the nitrogen of nitrates.[2] The insoluble nitrogen was obtained by subtracting the water-soluble nitrogen from the total nitrogen of the leaves. The various forms of nitrogen—such as the water soluble, nitrate, free amino acid, and ammonia—were obtained from the aqueous extract of the leaves in the following way:

A 10-gram sample of the powdered leaves was placed in a bottle containing 500 cubic centimeters of distilled water and shaken in a shaking machine for two hours. After the bottle had been set aside to allow the powder to subside, the clear supernatant liquid was carefully decanted from the sediment. The treatment was repeated twice, with the same amount of distilled water. The combined aqueous liquids were filtered, and the wet powder from the bottle was poured into the filter. The filtered aqueous extract was made to a volume of 2 liters.

Water-soluble nitrogen.—Two 100-cubic-centimeter portions of the aqueous extract were transferred to Kjeldahl digestion flasks and concentrated to about 30 cubic centimeters. The nitrogen in the concentrated solution was determined in accordance with the method suggested by Pucher, Leavenworth, and Vickery for the total nitrogen of plant extracts in the presence of nitrates.[3]

Nitrate nitrogen.—This was determined in an aliquot portion (150 cubic centimeters) of the aqueous extract, using Devarda's alloy method modified by Whiting, Richmond, and Schoonover.[4]

[2] Official and tentative methods of analysis of the Assoc. Offic. Agr. Chemists. (Revised to July 4, 1924.)

[3] Industrial and Engineering Chemistry, Analytical Edition 2 No. 2 (1930) 191.

[4] Industrial and Engineering Chemistry 12 No. 10 (1920) 982.

Free amino acid nitrogen.—Two aliquot portions of 50 cubic centimeters of the aqueous extract were evaporated on a water bath to about 10 cubic centimeters and used for the determination according to Van Slyke's method.[5]

Ammonia nitrogen.—This determination was made on an aliquot portion (150 cubic centimeters) of the aqueous extract according to a method which was essentially the same as that described by Longi.[6] In this method, the solution is rendered alkaline with magnesium oxide, a few drops of caprylic alcohol are added to prevent excessive foaming, and the mixture distilled in vacuo at a temperature of 38 to 40° C.

The acid amide, humin, basic, and nonbasic nitrogen of the aqueous extract were determined according to the method of Hausmann as modified by Jodidi.[7]

The nitrogen partition in the leaves after acid hydrolysis was carried out according to Jodidi and Moulton's procedure.[8]

The nitrogen distribution in the water-insoluble portion of the leaves was ascertained by subtracting the different forms of nitrogen obtained in the aqueous extract of the leaves from the corresponding forms of nitrogen determined in the leaves after acid hydrolysis.

DISCUSSION OF RESULTS

The total nitrogen in the leaves of the camphor trees as shown in Table 1 varies according to the age of the leaf. This variation is also observed in the distribution of the different forms of nitrogen as can be seen in Tables 2 to 5. There is, however, no consistent variation in any certain direction.

The old leaves have a higher free amino nitrogen (Tables 2 and 3) but are lower in humin nitrogen (Tables 4 and 5) than either the young or full-grown leaves. The young leaves, on the other hand, have lower ammonia nitrogen than either the full-grown or old leaves (Tables 2 and 3). With respect to other forms of nitrogen, no significant differences can be noted. The exceedingly high percentage of nitrogen from free amino acids in the old leaves may be explained as due to the rapid hydrolysis of the protein so that the amino acids can be translocated to the stem and other growing parts of the plant. According to

[5] Journ. Biol. Chem. 12 (1912) 277.
[6] Landw. Vers. Stat. 32 (1886) 15.
[7] Journ. Am. Chem. Soc. 42 (1920) 1883.
[8] Journ. Am. Chem. Soc. 41 (1919) 1526.

Combes,[9] before the leaves turn yellow most of the nitrogenous constituents are withdrawn.

A consideration of the entire experimental data shows that, in general, no consistent differences were found in the different forms of nitrogen which occur in the leaves of trees containing a mixture of camphor crystals and in the leaves of those which yield only a lævo oil.

TABLE 1.—*Total nitrogen in the leaves of Philippine camphor trees.*

Tree No.	Location.	Kind of leaves.	Plant product from leaves (camphor crystal or lævo oil).	Nitrogen.[a]
				Per cent.
8–F	Near Teacher's Camp	Young	Oil	3.31
8–F	do	Full-grown	do	1.66
8–F	do	Old	do	1.69
8–E	do	Young	Crystal	2.44
8–E	do	Full-grown	do	2.56
8–E	do	Old	do	2.05
19	Near office, Bureau of Forestry	Young	Oil	1.50
19	do	Full-grown	do	1.96
19	do	Old	do	2.18
19–A	do	Young	Crystal	2.72
19–A	do	Full-grown	do	1.45
19–A	do	Old	do	2.07
12–G	Session Road near Military Circle	Full-grown	Oil	1.87
12–D	do	do	Crystal	1.33
11	Forest Nursery, below Ranger's House	Old	Oil	1.37
11–A	do	do	Crystal	1.48

[a] Calculated on moisture- and camphor-free basis.

SUMMARY

1. The total nitrogen and the distribution of its different forms in camphor leaves growing at Baguio, Mountain Province of the Philippines, vary according to the age of the leaf.

2. The old leaves showing signs of chlorophyll degradation have higher free amino acid nitrogen but lower humin nitrogen than either the young or full-grown leaves. On the other hand, the young leaves have lower ammonia nitrogen than either the full-grown or old leaves. Other forms of nitrogen show no significant differences.

3. No consistent differences appear to exist in the forms of nitrogen obtained from leaves of trees containing a mixture of camphor crystals and oil and from the leaves of those yielding only a lævo oil.

[9] Rev. gen. bot. 38 (1926) 430, 510, 565, 632, and 673.

TABLE 2.—*Nitrogen partition in the leaves of Philippine camphor trees.*
(Percentage based on moisture- and camphor-free basis.)

Tree No.	Kind of leaves.	Plant product from leaves (camphor crystal or lævo oil).	Total nitrogen.	Kind of nitrogen.								
				Water-soluble.	Insoluble.	Nitrate.	Free amino.	Ammonia.	Water-soluble.			
									Acid amide.	Humin.	Basic.	Nonbasic.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8-F	Young	Oil	3.31	0.45	2.80	0.12	0.07	0.02	0.07	0.05	0.07	0.26
8-F	Full-grown	do	1.66	0.41	1.15	0.18	0.03	0.11	0.04	0.07	0.05	0.25
8-F	Old	do	1.69	0.30	1.39	0.10	0.10	0.07	0.03	0.02	0.03	0.22
8-E	Young	Crystal	2.44	0.29	2.15	0.15	0.06	0.04	0.05	0.03	0.03	0.18
8-E	Full-grown	do	2.56	0.36	2.20	0.11	0.02	0.08	0.03	0.07	0.06	0.20
8-E	Old	do	2.05	0.62	1.43	0.16	0.15	0.15	0.07	0.05	0.08	0.42

271104—11

TABLE 3.—*Nitrogen partition in the leaves of Philippine camphor trees.*
(Data expressed as percentage of the total nitrogen of the leaves.)

Tree No.	Kind of leaves.	Plant product from leaves (camphor crystal or lævo oil).	Kind of nitrogen.								
			Water-soluble.	Insoluble.	Nitrate.	Free amino.	Ammonia.	Water-soluble.			
								Acid amide.	Humin.	Basic.	Nonbasic.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8 F	Young	Oil	13.59	86.41	3.62	2.11	0.60	2.11	1.51	2.11	7.85
8-F	Full-grown	do	24.69	75.31	10.84	1.81	6.62	2.41	4.22	3.01	15.06
8-F	Old	do	17.75	82.25	5.96	5.91	4.14	1.78	1.18	1.78	13.01
8-E	Young	Crystal	11.88	88.12	6.14	2.45	1.63	2.04	1.22	1.22	7.14
8-E	Full-grown	do	14.06	85.94	4.29	0.78	3.11	1.17	2.73	2.34	7.82
8-E	Old	do	30.24	69.76	7.81	7.31	7.31	3.41	2.43	3.90	20.48

Tree No.	Kind of leaves.	Plant product from leaves (camphor crystal or lævo oil).	Kind of nitrogen.							
			Acid amide in—		Humin in—		Basic in—		Nonbasic in—	
			Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8–F	Young	Oil	0.38	11.42	0.22	6.74	0.29	8.67	2.42	73.17
8–F	Full-grown	do	0.28	16.80	0.28	16.68	0.24	14.52	0.86	51.98
8–F	Old	do	0.22	12.72	0.09	5.38	0.16	9.47	1.22	72.42
8–E	Young	Crystal	0.18	7.54	0.28	11.43	0.30	12.25	1.68	68.77
8–E	Full-grown	do	0.18	7.15	0.31	12.07	0.25	9.84	1.82	70.94
8–E	Old	do	0.24	11.75	0.18	8.68	0.23	11.07	1.40	68.48

[a] Percentage calculated on moisture- and camphor-free basis.

[b] Percentage based on the total nitrogen in the leaves.

TABLE 5.—*Nitrogen partition in the water-insoluble portion of the leaves of Philippine camphor trees.*

Tree No.	Kind of leaves.	Plant product from leaves (camphor crystal or lævo oil).	Kind of nitrogen.							
			Acid amide in—		Humin in—		Basic in—		Nonbasic in—	
			Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]	Leaves.[a]	Total nitrogen.[b]
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8-F	Young	Oil	0.31	9.31	0.17	5.23	0.22	6.56	2.16	65.32
8-F	Full-grown	do	0.24	14.39	0.21	12.46	0.19	11.51	0.61	36.92
8-F	Old	do	0.19	10.94	0.07	4.20	0.13	7.69	1.00	59.41
8-E	Young	Crystal	0.13	5.50	0.25	10.21	0.27	11.03	1.46	61.63
8-E	Full-grown	do	0.15	5.96	0.24	9.34	0.19	7.50	1.62	63.12
8-E	Old	do	0.17	8.34	0.13	6.25	0.15	7.17	0.98	48.00

[a] Percentage calculated on moisture- and camphor-free basis.

[b] Percentage based on the total nitrogen in the leaves.

PSEUDOMYCETOMA IN THE PHILIPPINES, WITH REPORT OF ONE CASE

By CARLOS MONSERRAT

Of the Department of Pathology and Bacteriology, College of Medicine University of the Philippines, Manila

FOUR PLATES

Previous to the acceptance of Chalmers and Archibald's classification, the term mycetoma was applied to all granulomatous conditions, often localized in the foot, caused by fungi, but without much consideration of the type of the causative microörganisms.

Carter(1) in 1860 studied cases of mycetoma of the foot, a disease prevalent in the town of Madura and recognized first the fungous nature of the infection.

Chalmers and Archibald(2) considered later as mycetomas the actinomycoses and the maduromycoses. The maduromycoses, although clinically similar to the actinomycoses, differ in respect to the microscopic appearance of their causative fungi.

The definition given of mycetoma is as follows: Mycetoma includes all growth and granulations in any of the tissues of men or animals which are caused by fungi belonging to different genera and species, which produce bodies or granules called grains of various dimensions, shape and colors composed of hyphæ and sometimes clamydospores which are found either embedded in the pathological tissues or escaping freely in the discharge therefrom.

In 1918 Chalmers and Archibald(3) described a condition of the foot which resembles more or less Madura foot in external characters, differing only in the absence of typical grains in the pus or tissues. As this condition appears to be of importance from a diagnostic point of view the disease was designated under the new name of pseudomycetoma.

The term pseudomycetoma was first used by Castellani(4) to indicate a peculiar condition of the foot in tertiary yaws. According to Breil, however, pseudomycetoma must be considered as a clinical entity separated from yaws. Cases of pseudomy-

cetoma have been registered according to this author in New Guinea under different native names and the disease resembles typical Madura foot without the presence of the characteristic grains in the pus.

As a contribution to the knowledge of this important group of mycosis and with the purpose of interesting tropical practitioners in the search for cases of this nature, I report a case registered in a native of the Philippines. The lesion of the foot resembles a typical Madura foot, as clearly seen by the picture. I consider this case a pseudomycetoma since the typical grains could not be demonstrated either in the pus in the several examinations or in the histological preparation of tissues removed in the biopsy. The bacteriological smears and cultures from the pus of the sinuses revealed, in the absence of other bacteria, the presence of a fungus belonging to the group of *Nocardia* or *Streptothrix*. The strain isolated was pathogenic to guinea pigs and monkeys, and when injected into a monkey's foot produced a swelling that was quickly followed by the formation of a suppurative lesion.

It is worth while to mention here that in reviewing the medical literature in the Philippines, our case seems to be the first one reported of this special type of mycetoma, since the only case studied in 1907 by Musgrave and Clegg was considered a true mycetoma with typical grains of the ochroid variety. At the same time it is interesting to say that the fungus isolated in our case resembles in many respects the organism described by Musgrave and Clegg under the name *Streptothrix freeri*. Very probably this particular strain is predominating in this country.

HISTORY

E. O., 28 years old, male, Filipino, married, born in San Narciso, Pangasinan Province, Philippine Islands, complained of painless enlargements of the left ankle with multiple nodules on the same. Family history negative. Previous history unimportant, except for this affection of the foot.

The present illness is of nine years duration, following a misstep after which the patient noticed a small elongated hard mass (1 by 3 centimeters) below the external malleolus of the left ankle. The mass was nontender, and movable with the skin adherent to it. This did not increase in size until four years ago, when the external surface of the left foot was caught by one of the teeth of a harrow, wounding the part. Following this, the mass at the external malleolus of the left ankle enlarged

rather rapidly, covering the entire malleolus. The wound suppurated and healed in a week's time, only to be followed by a small elongated mass underneath the skin beside the scar. The mass then softened and later burst, giving out pus and dead blood. Around the first mass several small elongated nodules developed, and thus the swelling and induration of the part spread peripherally outward. At times several hard nodules developed and ruptured, and at other times only one developed. The time of rupturing varied from a day to a week. This process continued until nearly the whole left foot and part of the ankle were involved but respecting so far the plantar region. There was also, besides the induration and the nodules, a dark discoloration of the skin.

Physical examination.—Nothing of importance except the localized lesion. The left ankle including most of the left foot is markedly enlarged, with multiple nodules on the surface. The swelling is slightly tender on very deep pressure. There are several nodular swellings all over the ankle and medial surface of the foot except on the plantar region, some hard and some fluctuating of about the size of mongo beans, exuding a thin seropurulent discharge. Some of these nodules are healed and scarred, others are covered by hyperkeratotic skin. No granules can be seen in the pus from these nodules. There is hyperpigmentation of the skin. The leg is enlarged up to the lower half but atrophied at the upper half.

Laboratory.—Blood examination gave the following:

Red blood cells		3,800,000
Hemoglobin (Talquist method), per cent		75
Leucocyte count		8,800
Differential:		
Neutrophils	75	
Small lymphocytes	17	
Large lymphocytes	4	
Large mononuclears	7	
Eosinophils	0	
	100	

Blood pressure, systolic, 148; diastolic, 52. Urine and fæces, nothing important. X-ray examination, slight changes of osteoperiostitis in lower end of left fibula. Wassermann blood test, negative. Temperature, afebrile throughout stay in ward.

Local antiseptic treatments were applied to the lesion, and potassium iodide was given per orem without improvement. Amputation of the foot was proposed, but the patient refused the

operation. After almost two weeks stay in the hospital a bacteriological culture from the lesion was made, the specimen being taken from a closed nodule. The material was planted on blood hormone agar slant tubes and incubated at room and incubator temperatures. On the fourth day after inoculation a pure growth of a streptothrix organism was obtained from the tubes and no other bacterial colony was present. The morphological, tinctorial, and biological characteristics of this organism are as follows:

Morphology.—Smears from young cultures show numerous filamentous branching forms. The filaments have a definite wall and some of the hyphæ show slight swollen ends. The organism stains well with ordinary aniline dyes and in these preparations the filaments show in the interior few coccoid and bacillary forms of various length. Branching occurs as lateral hyphæ from the segmented portions. With the Ziehl-Neelson acid-fast stain method, the bacillary forms in young cultures assume the bright red color of tubercle bacilli but the filaments are as a rule non-acid fast; a few of them, however, are slightly stained pinkish. In old cultures, at least 7 days old, more numerous and deeply stained acid-fast filaments are found, also numerous coccoid and bacillary acid-fast forms. With the Gablet's acid-fast stain method more uniform and intensively acid-fast forms are obtained.

Cultures.—The streptothrix was cultivated without difficulty from the nodules and sinuses of the affected foot on hormone blood agar slant tubes. Visible growth appeared on the second day, and typical and good growth was obtained on the fourth day. The isolated colonies on this medium were dry, rounded, somewhat smaller than the staphylococcus colony, with folded edges and slightly umbilicated centers, simulating small rosettes. The colony was pale yellowish, turning yellowish pink with age. Later, more colonies developed and a heaped up and wrinkled yellowish pink growth was obtained. No hæmolysis was noticed, but the medium became darker with age.

Transplants were made to peptone broth, litmus milk, potato, Loeffler's blood serum, ordinary agar, glucose agar, glycerin agar, glycerin broth, Saboraud's medium, and various carbohydrates.

A profuse growth appeared in a few days in certain of these media, in others the development was much slower. Glycerin agar, glucose agar, and hormone blood agar are considered the

best media. The growth was slightly more rapid at incubator temperature (37° C.) than at room temperature (28° C.).

Peptone water.—Good growth was obtained after two or three days only on the surface of the medium, as small, delicate, pale yellowish, flat particles slightly umbilicated in the center which became confluent and adherent to the sides of the tubes. The medium did not become cloudy. The so-called puffball growth was not observed.

Litmus milk.—Moderate growth was obtained after two or three days on the surface of the medium, of the same character as in peptone broth. No change of color occurred in the medium, but in time the upper part of the culture, below the surface growth, became darker blue. Milk was not coagulated.

Potato.—Very scanty growth was obtained in the medium even after four days incubation, in the form of minute yellowish white colonies.

Loeffler's blood serum.—Growth appeared after three days in the form of minute, dry, confluent, yellowish colonies, which later became yellowish pink. There was no evidence of liquefaction.

Agar —1 per cent.—Good growth was obtained after three days as a powdery, confluent, whitish yellow growth.

Agar +1 per cent.—Scanty growth was obtained after three days as small, dry, yellowish colonies with slightly umbilicated center.

Glucose agar.—In slant tubes good growth was obtained after two or three days as confluent, minute, dry, yellowish colonies. In a week a heavy heaped up growth was observed with pronounced pinkish coloration. In stab tubes a wrinkled heaped up yellowish pink growth was obtained on the surface, and no growth on the depth of the agar.

Glycerin agar.—A confluent, heavy, heaped up growth was obtained in two or three days. Colonies were small, dry, rounded, umbilicated, and whitish in color. Later colonies turned yellowish and further yellowish pink.

Carbohydrates.—Subplants on lactose, dextrose, maltose, saccharose, and mannite broth with Andrade's indicator, showed surface growth the same as in peptone. Neither acid nor gas fermentation was noticed on prolonged incubation.

Saboraud's medium.—Good growth was obtained at room temperature in the form of a powdery whitish growth which did not become pinkish.

Pathogenicity.—A monkey was injected with 2 cubic centimeters of a suspension of our organism in sterile saline; the injection was made in the tissues of the right foot, the foot became inflamed and an ulcer developed after three days which discharged a thick purulent material. The streptothrix was recovered in the cultures from the lesion. A guinea pig was injected intraperitoneally with thick streptothrix suspension in sterile saline. The animal was killed thirteen days after injection. Autopsy revealed very minute necrotic yellowish nodules in the peritoneal surface, omentum, and liver. Cultures from different nodules yielded pure growth of streptothrix.

Histological sections from liver, stained with hæmatoxylin eosin, showed small rounded necrotic areas containing polymorphonuclear leucocytes with the fungus at the center. The organisms in section stained well and resembled in appearance the granule of actinomyces. At the periphery the radiating mycelial threads could be seen, but the ray formation and the club appearance were much less noticeable.

SUMMARY

The disease known as mycetoma, or Madura foot, was diagnosed formerly on clinical grounds, without much regard to the type of the infecting fungus. The finding of the typical grain in the pus or tissues is considered the most important in the diagnosis. Three varieties of grains were recognized; namely, the black, the red, and the ochroid or yellow, the last being the commonest found.

Chalmers and Archibald(2) introduced more recently the clinical terms pseudomycetoma and paramycetoma to differentiate from true mycetoma conditions of the foot clinically similar to Madura foot, and also certain sarcomalike and epitheliomalike tumors, caused by fungi in which the characteristic grains cannot be easily demonstrated, probably due to their reduced numbers or absence.

From the bacteriological point of view, there is difference of opinion in regard to the etiology of Madura foot. Some authors believe that the disease is caused by actinomyces, others by different types of streptothrix organisms. The first contention, however, is not well supported in the few cases reported in the literature.

Kanthack(5) in 1893 studied three cases of the black type and twelve of the yellow. He concluded that both were due to the same fungus and actinomyces.

Boyce and Surveyor(6) in 1894, while acknowledging the similarity of the organism found in these two varieties of mycetoma and in actinomycosis, believed that the two forms differ distinctly in etiology.

Vincent(7) in 1894 reported a case of the ochroid variety and cultivated a streptothrix from the lesions. Vincent considered his microörganism different from actinomyces not only morphologically but also in cultures and in inoculation experiments. This microörganism was named *Streptothrix maduræ.*

Wright(8) in 1894 succeeded in cultivating a new variety of streptothrix from a case of mycetoma of the black type. The work of Vincent and Wright seem to be carefully carried out and their conclusions appear to be sound.

Foulerton and Jones in 1902 (cited by Musgrave and Clegg) published an exhaustive article dealing with streptotricosis in general. The authors in discussing mycetoma considered *Streptothrix maduræ* Vincent the cause of the ochroid variety and Wright's streptothrix the cause of the black type.

McLeod(9) believes that Vincent's streptothrix is the etiologic agent of the yellow variety of mycetoma, and that this organism is allied to but differs from the actinomycosis. The black species of Madura foot, he thinks, is due to a degenerated form of the same fungus.

One of the most valuable investigations regarding the etiology of mycetoma is that of Musgrave and Clegg(10) in 1907. These authors reported a typical case of Madura foot of the ochroid variety of three years duration in a Filipino woman, 30 years old. A new variety of streptothrix was isolated from the lesions and the microörganism was named *Streptothrix freeri.* With this organism they performed inoculated experiments on forty monkeys, guinea pigs, rabbits, dogs, and pigeons, and in three instances typical examples of Madura foot developed in monkeys after the injection of cultures in the tissues of the foot. These investigators believed at the same time that actinomycosis has been mistaken for Madura foot, both of the ochroid and black types in some of the reported cases, and concluded that it is probable that all types of mycetoma are due to streptothrix infection. Whether all forms are caused by the same organism or whether more than one species plays a part in the disease can not be stated positively.

Quite recently, in 1916, Clegg and Hobdy(11) reported another case of Madura foot, also of the ochroid variety, in a native woman of Hawaii, of five years duration. The microörganism

isolated is a streptothrix culturally resembling *Streptothrix maduræ* of Vincent.

Our case is interesting both from the clinical and bacteriological standpoint. Clinically, the lesion resembles typical Madura foot, but the characteristic grains could not be demonstrated in the pus of the sinuses and in the histological sections of the biopsy performed. Bacteriologically, the fungus isolated is an acid-fast, pathogenic, branching streptothrix differing in these peculiar features from Wright's streptothrix isolated in the United States from a case of Madura foot of the black type, and from Vincent's and Clegg and Hobdy's streptothrix isolated respectively in India and Hawaii from cases of the ochroid variety. Our organism resembles in acid-fast properties, pathogenicity, and other biological features the fungus described by Musgrave and Clegg as *Streptothrix freeri*, isolated from a case of Madura foot of the ochroid type which occurred also in a native of the Philippines.

BIBLIOGRAPHY

1. Carter, H. Vandyke. On mycetoma or the fungus disease of India. London, Churchill (1874) 118.
2. Chalmers, A. J., and R. G. Archibald. The classification of mycetomas. Journ. Trop. Med. and Hyg. 21 (1918) 121.
3. Chalmers, A. J., and R. G. Archibald. Paramycetoma. Journ. Trop. Med. and Hyg. 21 (1918) 177.
4. Cited by Castellani and Chalmers. Manual of Tropical Medicine 3d ed., p. 2147.
5. Kanthack. Journ. Path. Bact. (1893) 140.
6. Boyce and Surveyor. Trans. Roy. Soc. London (1894).
7. Vincent. Am. Inst. Pasteur (1894) 129.
8. Wright. Journ. Exp. Med. 3 (1898) 421.
9. McLeod. Allbutt's System of Medicine 2d ed. 2 (1907) 754.
10. Musgrave and Clegg. Philip. Journ. Sci. § B 2 (1907).
11. Clegg and Hobdy. Am. Journ. Trop. Dis. and Prev. Med. 3 (1916) 534.

CHARACTERISTICS OF STREPTOTHRIX AS REPORTED BY VARIOUS AUTHORS

STREPTOTHRIX OF WRIGHT (UNITED STATES)

Morphology.—Long branching filaments, 3 to 8 microns in diameter. In young forms delicate transverse septa are present. In older forms there may be swelling at these points and the hyphæ may appear as a string of oval-ended plump segments. The filaments have a definite wall, and in the interior granules or pale areas may be seen. The branching occurs by the formation of lateral outgrowth of buds. No spore bearer organisms were observed.

Acid-fast properties.—Not acid fast.

Peptone broth.—Growth always proceeds from the planted material whether grain or fragments of mycelium in the form of fine radiating filaments and soon produces a puffball appearance. Eventually the whole area of fluid is filled with radiating mycelium and a definite mycelial layer is formed on the surface. The medium acquires a coffee-brown color.

Potato.—It forms a dense widely spreading coherent membrane or layer of velvety surface, pale brown in color throughout its central portion and white at its peripheral portion. A marked feature is the appearance of small spherical globules or droplets of dark coffee-colored fluid on the surface of this layer. The potato takes a dark brown color and becomes moist.

Glucose agar.—The growth appears as a meshwork of widely spreading filaments of a grayish color on the surface of medium. In old cultures black sclerotia are found as in potato infusion. In stab cultures growth appears only on the surface.

Pathogenicity.—No results were obtained from the inoculation of animals with the original granules or with cultures.

STREPTOTHRIX OF VINCENT (INDIA). DESCRIPTION OF MUSGRAVE AND CLEGG AND OF VINCENT AND FOULERTON

Morphology.—Long thin filaments with true branching: no club forms. These hyphæ may or may not be radially placed at the periphery of the granule. (Musgrave and Clegg.)

Acid-fast properties.—Cultures stained by Ziehl-Neelsen method showed no acid-fast portions; Gram positive with irregular staining. (Musgrave and Clegg.)

Peptone broth.—Growth appears as opaque white globular, more or less cohering colonies at bottom of tube; surface growth rare. No pigmentation of medium. (Vincent and Foulerton.)

Alkaline-litmus milk.—Growth occurs. No obvious change happens in the milk until after twenty days incubation, the medium then becomes pink and after four weeks begins to clear. (Vincent and Foulerton.)

Potato.—Growth appears after three days as small pinkish white colonies, which later develop a dark pink round granular growth. (Musgrave and Clegg.)

Loeffler's blood serum.—Growth slow and scanty. No liquefaction or pigmentation of medium. (Vincent and Foulerton.)

Glycerin agar.—Growth appears after five days as small, pinkish granules and later assumes a dark pink color. (Musgrave and Clegg.)

Resistant to heat, drying; relative growth to temperature; thermal death point.—Sporulating cultures resist an exposure to 45° C. for thirty minutes but are killed by exposure to 60° C. for the same period. (Vincent and Foulerton.)

Pathogenicity.—Inoculation of cats, rabbits, guinea pigs, and mice produced nothing more than a small nodule at the site of inoculation and these disappeared. (Vincent.)

STREPTOTHRIX MUSGRAVE AND CLEGG (PHILIPPINE ISLANDS)

Morphology.—Long branching filaments 2 to 7 millimeters in diameter. Transverse segments are shown of various lengths from coccoid to 10 μ length. Branching occurs as lateral hyphæ developing from the segments. The filaments have a definite wall. Spores have not been observed. Coccoid and clublike forms present in certain media.

Acid-fast properties.—Many acid-fast portions in cultures from 5 days to 2 months old (Ziehl-Neelsen method).

Peptone broth.—Growth occurs after three days, as flat particles on the surface of the medium. These produce in time a delicate pink color and when the tube is shaken they adhere to its sides. As the growth proceeds a granular mass collects at the bottom of the tube. This mass is more or less coherent. Medium does not become cloudy but in time becomes darkened.

Alkaline litmus milk.—Growth appears after three days on surface of medium, as flat drying appearing particles. These later become confluent, forming a heaped up pinkish yellow mass. No reaction occurs in the medium until after the second week, when the color gradually fades but does not become red. This decolorization begins at the bottom. Milk is not coagulated.

Potato.—Growth appears as small pinkish colonies, later raised and becoming confluent. After a few days the growth assumes a heaped up appearance resembling a mass of yellow curls. Medium becomes moist and dark.

Loeffler's blood serum.—Growth appears, after three days, smooth and delicate pink. Growth does not change the character of the medium after three weeks incubation.

Glucose agar.—Growth appears after three days; later becomes luxuriant with a heaped up and wrinkled appearance. The center becomes pinkish yellow with a gradual elimination to a delicate pink and to a white periphery. Stab cultures show only surface growth, with the same pigment characteristics.

Glycerin agar.—Grows well, appearing after three days as raised heaped up moist growth; produces a yellow pigment in center with a pale periphery.

Resistant to heat, drying; relative growth to temperature; thermal death point.—Is killed by exposure to 70° C. for fifteen minutes.

Pathogenicity.—Intraperitoneal inoculations of guinea pigs, monkeys, and dogs with material from lesions and from cultures in nearly every case produced processes resulting in death after two weeks. Rabbits and pigeons were not affected other than by the production of small nodules at site of inoculations in subcutaneous injection; these soon underwent resolution and healed. No progressive lesions were produced by subcutaneous inoculations. In monkeys inoculated in the foot with cultures extensive lesions were produced anatomically resembling Madura foot. Pure cultures were obtained in every instance from experimental lesions.

STREPTOTHRIX C. MONSERRAT (PHILIPPINE ISLANDS)

Morphology.—Smears from young cultures show numerous filamentous branching forms; some of the hyphæ are slightly swelled at the ends.

Filaments have definite wall and in stain preparations show in the interior few coccoid and bacillary forms of various lengths. Branching occurs as lateral hyphæ from the segmented portions.

Acid-fast properties.—In young cultures the bacillary forms are acid fast but filaments as a rule are non-acid fast. In cultures 7 days old more acid-fast filaments are found and numerous bacillary and coccoid acid-fast forms. With Gabbet's stain, acid-fast forms are more deeply and uniformly stained.

Peptone broth.—Good growth obtained after two to three days on the surface of the medium in the form of small, rounded, delicate, flat particles slightly umbilicated in the center which in time become confluent, forming a heaped up whitish yellow pellicle. On being shaken these particles adhere to the side of the tube. The medium does not become cloudy. The puffball growth was not obtained.

Alkaline litmus milk.—Moderate growth is also obtained after two to three days on the surface of the medium as small, rounded, flat particles which become confluent, the same as in peptone broth. No change of color occurs in the medium, but in time the upper part of the medium below the surface growth becomes deeper blue. Milk is not coagulated.

Potato.—Scanty growth is obtained after four days in the form of minute yellowish white colonies.

Loeffler's blood serum.—Growth appears after three days in the form of minute, confluent, yellowish colonies, which later become slightly pink. No evidence of liquefaction.

Glucose agar.—Glucose agar slant growth is obtained after two or three days in the form of confluent, minute, yellowish colonies. In a week a heavy heaped up growth is obtained with a pronounced pinkish coloration. In stab tubes growth is obtained only on the surface and not in the depth of the agar.

Glycerin agar.—Confluent heaped up growth, obtained after two to three days, consists of rounded, slightly umbilicated, whitish colonies, which later becomes yellowish and further yellowish pink.

Pathogenicity.—A monkey was injected in the tissues of the foot. The foot became inflamed and an ulcer developed after three days which discharged thick purulent material. The streptothrix was recovered from the lesion. A guinea pig was injected intraperitoneally and was killed thirteen days after inoculation. Autopsy showed minute necrotic nodules on the peritoneal surface, omentum, and liver. Pure streptothrix culture was recovered from the lesions.

STREPTOTHRIX CLEGG AND HOBDY (HAWAII)

Morphology.—Long, thin, branching filaments. Spore-bearing organisms were not observed.

Acid-fast properties.—Not acid fast.

Peptone broth.—Growth appears as opaque white colonies at the bottom of the tubes, more or less coherent. The so-called puffball growth was not observed. On shaking the tube numerous flat flaky particles adhere to the sides. The microörganism grows as long, thin, branching filaments. The medium remains clear.

Alkaline litmus milk.—Nothing recorded.

Potato.—Nothing recorded.

Loeffler's blood serum.—Nothing recorded.

Glucose agar.—Growth was luxuriant after six days with heaped up mealy appearance. Old cultures developed a rich cream color. Bacillary and coccoid forms did not occur in this medium.

Glycerin agar.—Growth similar to that on maltose and glucose agar.

Resistant to heat, drying; relative growth to temperature; thermal death point.—Nothing recorded.

Pathogenicity.—Not pathogenic for guinea pigs, rabbits, or monkeys.

ILLUSTRATIONS

PLATE 1

FIG. 1. The patient's foot, markedly enlarged, with multiple nodules on the surface containing seropurulent discharge.

2. Smear from an old culture stained by Gabbet's acid-fast stain method, shows filamentous branching acid-fast forms with bacillary and coccoid forms inside of hyphæ.

PLATE 2

FIG. 1. Section of a liver nodule in a guinea pig injected intraperitoneally with our streptothrix, shows a necrotic area with polynuclear infiltration and the fungus at the center. (Hæmatoxylin eosin stain; high power.)

2. Section from the liver of a monkey injected intraperitoneally with our streptothrix. High-power view of a necrotic nodule with the fungus at the center and polynuclear cells.

PLATE 3

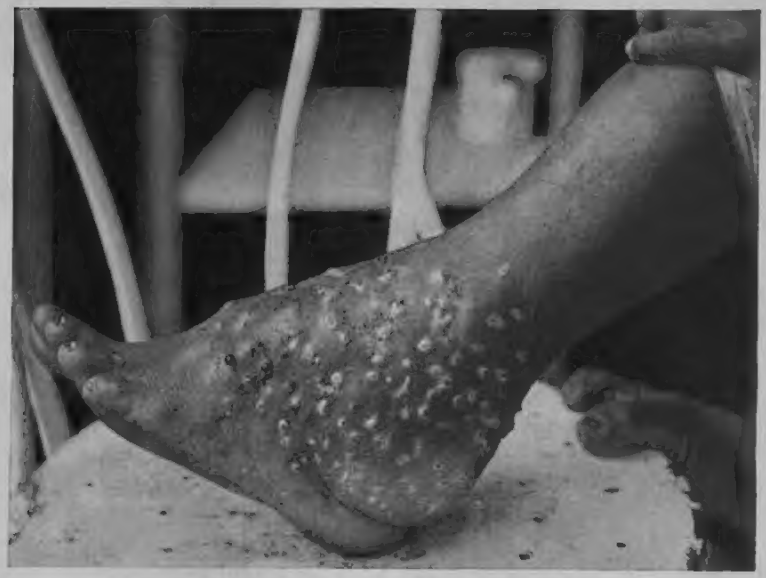
Culture in glycerin broth 3 weeks old; a confluent wrinkled yellowish surface growth.

PLATE 4

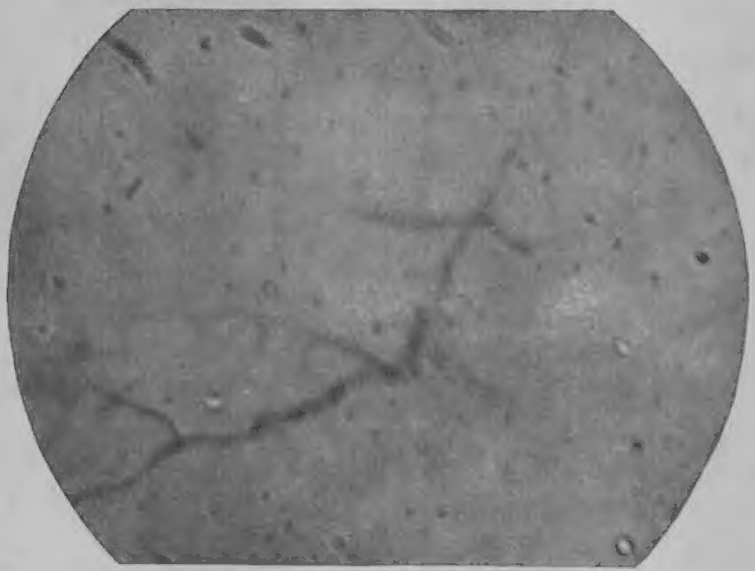
FIG. 1. Young culture on hormone blood agar, recovered from guinea-pig nodule. Animal inoculated with subplant of original strain, shows rosette ocher yellow colonies, umbilicated. (Cultures 5 days old.)

2. Original pure culture obtained from patient's foot, growing on hormone blood agar; ocher yellow colonies become confluent and acquire a pinkish coloration with age. (Cultures 24 days old.)

3. Old culture (23 days old) on Saboraud's medium, shows powdery whitish growth colonies which never become yellow or pink.

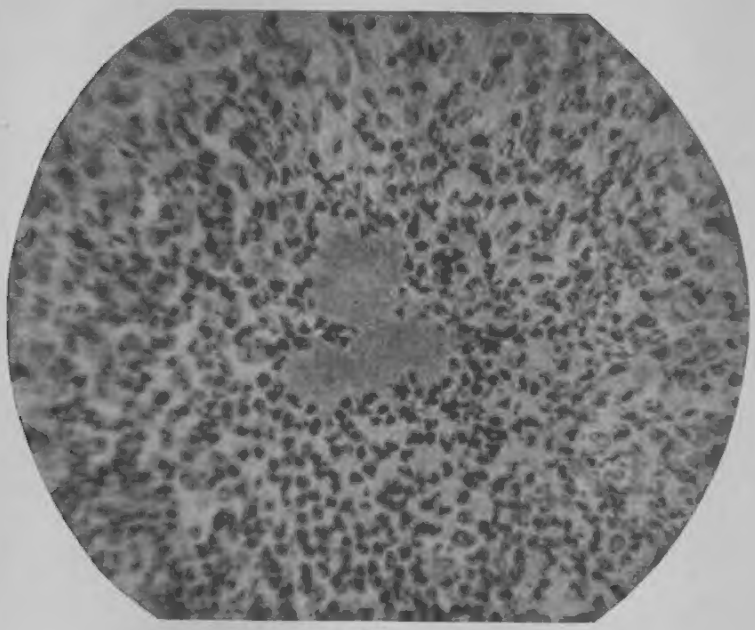


1

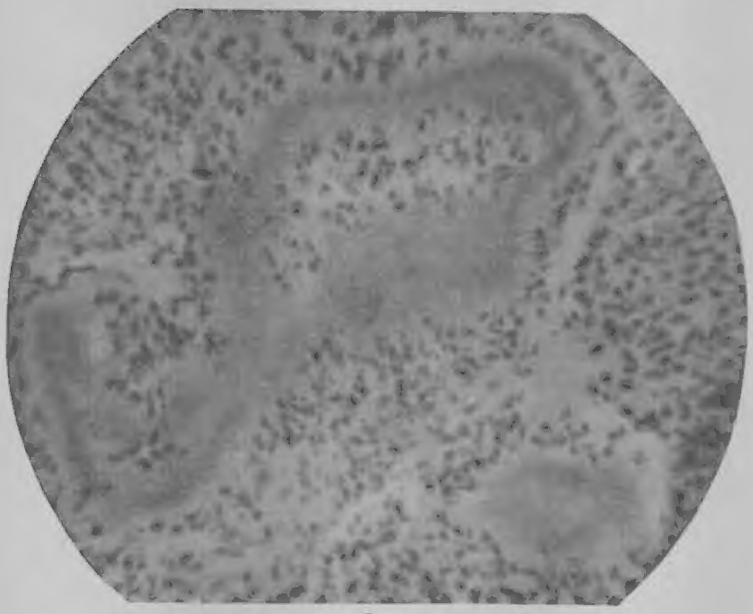


2

PLATE 1.



1



2

PLATE 2.

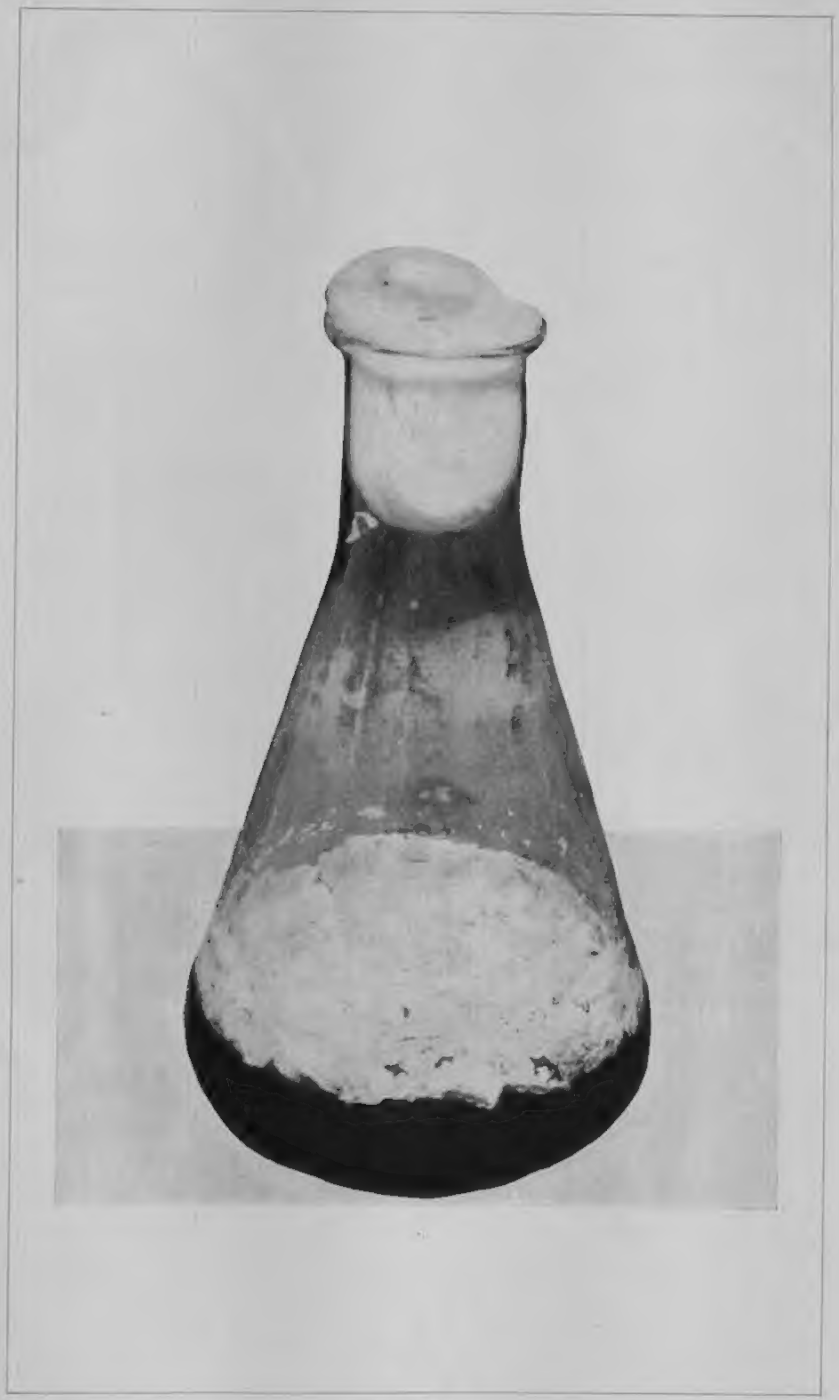


PLATE 3.

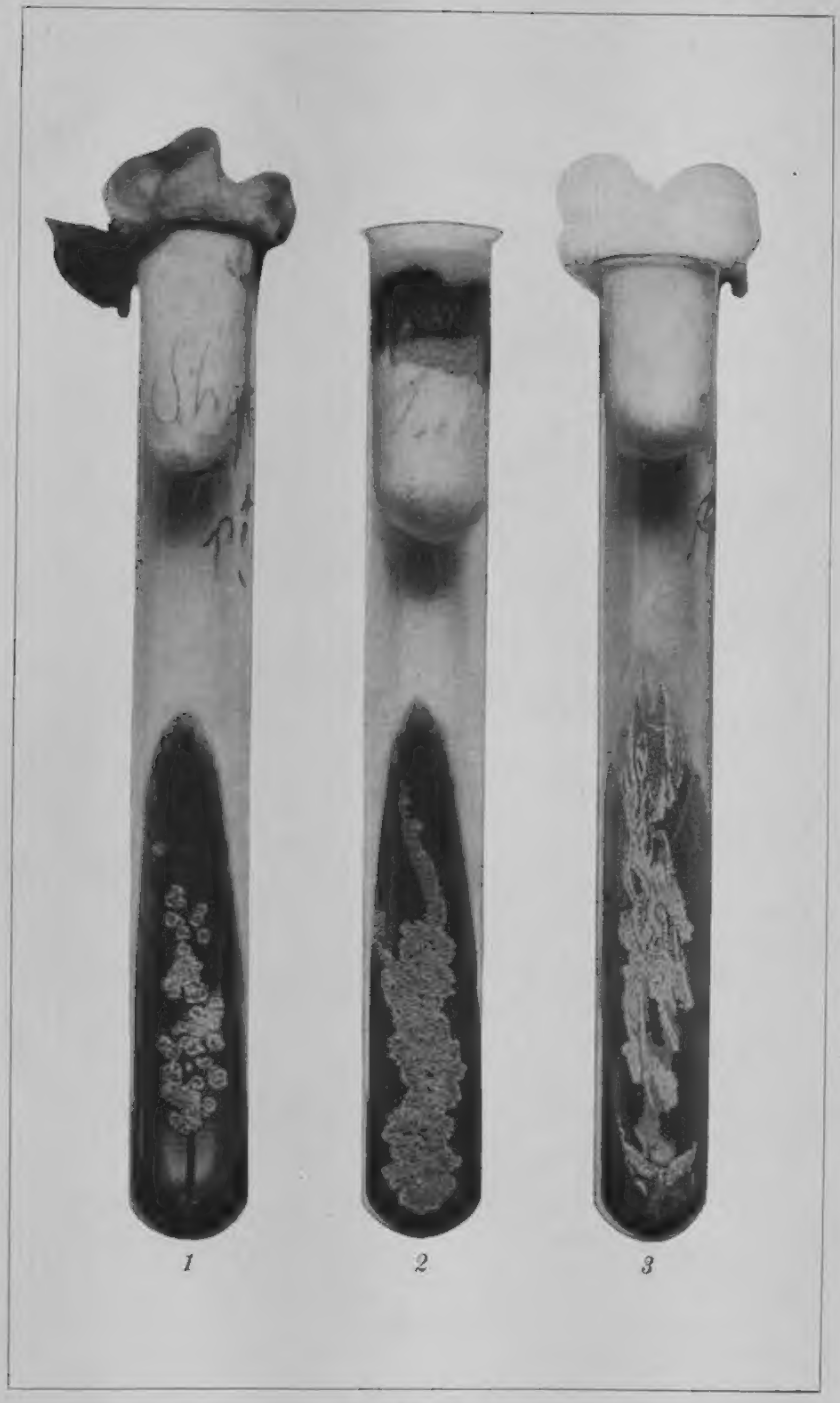


PLATE 4.